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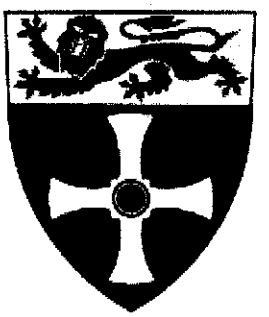

School of Chemical Engineering and Advanced Materials

MSc Sustainable Engineering

Research Project

'Integration of the Vegetable Oil Extraction and Biodiesel Production Processes'

By

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ABSTRACT

Biodiesel is a sustainable, low polluting fuel which can replace current diesel fossil fuel without changing the design of the combustion engine. With an aim to counteract the global warming, an integrated and intensified way of producing biodiesel needs to be introduced. Transesterification is a common method to produce biodiesel through reaction of alcohol (typically methanol and ethanol) with vegetable oil in the presence of catalyst. Vegetable oil is extracted from raw materials such as rapeseeds, soybeans or sunflowers and then refined before reaction takes place. This method however would incur high cost of production due to expensive feedstock and hence a shortcut way was investigated by introducing in-situ transesterification method, which eliminates refining stages.

By using rapeseeds at specified amounts, different type of alcohols, different type and strength of catalysts and varied ratios of alcohol to hexane (vol%), biodiesel was successfully produced using in-situ method. This method was feasible to produce high degree of conversion and large amount of biodiesel and therefore has high probability to be commercialised. The highest conversion achieved was 92%, with approximately 11.2 g of biodiesel was yielded through combination of 0.1 m sodium hydroxide in methanol and 30g of rapeseeds

Re-extraction was carried out to investigate the theory that small amount of alcohol will not produce biodiesel because it will be adsorbed by the rapeseeds. This was disproved since there was no methanol recovered during re-extraction. Instead, biodiesel was adsorbed on the surface and inside the seeds.

Emulsion liquid membrane (ELM) is a way to allow hexane extract the triglycerides and methanol would react with the triglycerides extracted once the emulsions break up. However, the creation was a failure. Finally, time study was carried out to investigate the biodiesel production within specified duration. Higher degree of conversion and large yield were expected for longer period of extraction and reaction. Thin layer chromatography was used to give qualitative and quantitative analysis, via image analysis software.

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1.0 INTRODUCTION

Biodiesel is a sustainable, low polluting fuel produced from various vegetable oils – principally rapeseed oil in Europe. To counteract the global warming problem, biodiesel can be used as an alternative fuel, which is environmentally friendly (no emission of SO_x), and has low carbon emissions (CO and CO_2). This statement is arrived at by considering the life cycle of the biodiesel, from the agricultural production of the biomass to the end-use, compared to conventional diesel.

Currently, the most important issue is the willingness of the manufacturers in producing biodiesel as an alternative fuel. The main factor that limits biodiesel production is the feedstock costs i.e., rapeseed oil, which results in higher operating cost compared to petroleum diesel fuel[1].

If the extraction of rapeseed oil from its crushed seed and the reaction of that oil (with methanol or ethanol) to biodiesel can be performed in one step, it is believed that the running and capital costs of biodiesel production can be reduced. For this reason, there are many small biodiesel companies who are already interested in the concept of producing their own biodiesel.

1.1 Aim and Objective

The aim of this project is to determine a method of achieving the integration and intensification of process steps in biodiesel production. One likely method is to use alcohol as both reactant and solvent. The next stage is to determine the optimal combination(s) of solvent, catalyst and alcohol. If the reaction is feasible and can be further exploited, the reactions hence will be performed in a novel reactor known as the 'oscillatory flow reactor', which is able to suspend small particles (the seeds in this case) in a controllable manner, whilst providing good liquid-liquid mixing between the different phases.

The specific objectives set in this experiment are:

1.1.1 To investigate the feasibility and optimal condition of in-situ transesterification through different types of alcohols and catalyst's strength.

1.1.2 To prove the previous theory stated that biodiesel was not produce since some amount of methanol was adsorbed by the rapeseeds.

1.1.3 To investigate the reaction's behaviour along specified reaction time.

1.1.4 To analyse the possibility of combining solvent for extraction and reagent for reaction through production of liquid membrane.

1.1.5 To design a better analytical method for biodiesel analysis.

1.2 Strategy

In order to achieve the listed objectives, a literature review is carried out to gain an understanding of biodiesel, its sustainability aspect, the production, and any recent development in biodiesel production. Experiments are designed and the conditions and the parameters to be varied are set. Most of the experiments are designed based on literature review so that most of the conclusions drawn are comparable with the literature highlighted.

2.0 LITERATURE REVIEW

The following is a discussion of the research that has been conducted on biodiesel production and sustainability. This review is important as a first step to start this project. It would definitely help in setting up the experiments, analysing and discussing the results obtained from the experiments. This review focuses on the sustainability of biodiesel production, distributed economies, carbon cycle of biodiesel, biodiesel processing which includes the kinetic of reaction and also the choice of catalysts. Other important aspects to be discussed here include the novelty of in-situ transesterification, biodiesel qualitative and quantitative analysis and also liquid membrane. Eventually, this discussion will arrive at intensified equipment, Oscillatory Flow Reactor (OFR), which can be used to process biodiesel and possibly through this novel route.

2.1 DISTRIBUTED ECONOMIES – A NEW ENGINE FOR SUSTAINABLE PRODUCTION

The concept of distributed economies (DE) is a fresh strategy to guide industrial development towards becoming more sustainable. According to Johannson *et al*[2], this concept is trying to depart from the socio-economically and environmentally unsustainable dynamics associated with large-scale production.

It is undeniable that growth which is predominantly driven by the sole rule of 'production efficiency' often results in centralised, large-scale production units. These units, mostly under the ownership of large and powerful corporations, have increasingly uniform product outputs that are mostly traded in global markets. However, the dominance of such centralised, large-scale production units causes dynamics that undermine sustainability.

According to Mirata *et al*, energy and biomass sectors, the productions themselves can be extended to operate at wider region. However, as for distributed system production, local orientation is applied. As an example, bio-energy sectors in Nordic countries employed decentralised solutions at the municipal level and utilised locally available

materials, which enabled increased value addition to local resources and has reduced the regional dependency on fossil-fuels.

Another system is the operation of bio-fuels based combined heat and power (CHP), located in Enköping, Sweden. The decision was to plant Salix trees as to replace part of their imported fuels by locally produced one. This alternative and reliable fuel source provides increased flexibility and the foundation for enhanced economic stability.

The main advantage of the distributed economies (DE) engine is flexibility of input, which leads to local economic stability. Apart from that, it encourages local commitment and collaboration, encourages local ownership and last but not least, reduces the environmental impact with the condition that it is a small scale production.

The aim of this project is to develop a process for small biodiesel companies where the production will be operated on site and surrounded by rapeseed plantation. This is believed to cause a reduction in carbon emissions over the life cycle of the biodiesel. Apart from that, distributed production will help to support the sustainability of biodiesel production socially and economically by encouraging local farmers to plant rapeseed oil and provide job opportunities for local people.

2.2 CARBON CYCLE FOR RAPESEED OIL BIODIESEL FUELS

According to Peterson *et al.*[3], replacing petroleum diesel with biodiesel could reduce the accumulation of CO_2 in the atmosphere. It has been estimated that, for rapeseed seedlings, 17-19% of the fixed CO_2 was translocated to the roots over a period of two weeks. 23-24% of that released to the atmosphere through respiration and 30-34% goes into rizosphere (soil). Of the rizosphere, 35-51% was used by micro-organisms. This would leave around 30-40% of the carbon translocated to the roots in the soil. Therefore, the plants take in more CO_2 than is accumulated in the plant biomass. Nonetheless, the carbon in the soil would degrade and finally releasing CO_2 in the atmosphere.

A theoretical rapeseed oil carbon cycle is depicted in figure 3.1. Harvesting rapeseed and converting it into rapeseed oil will release considerable amounts of CO_2 to the atmosphere. Rapeseed oil, rapeseed, rapeseed meal, and rapeseed biomass yield between 2450 and 8500 kg CO_2 per hectare of rapeseed plants where rapeseed biomass or residue produced the highest amount of CO_2 in the atmosphere. Rapeseed residue, especially, remained in the rizosphere where it was metabolised by micro organisms, retaining some but returning the rest back into the atmosphere as CO_2 via respiration.

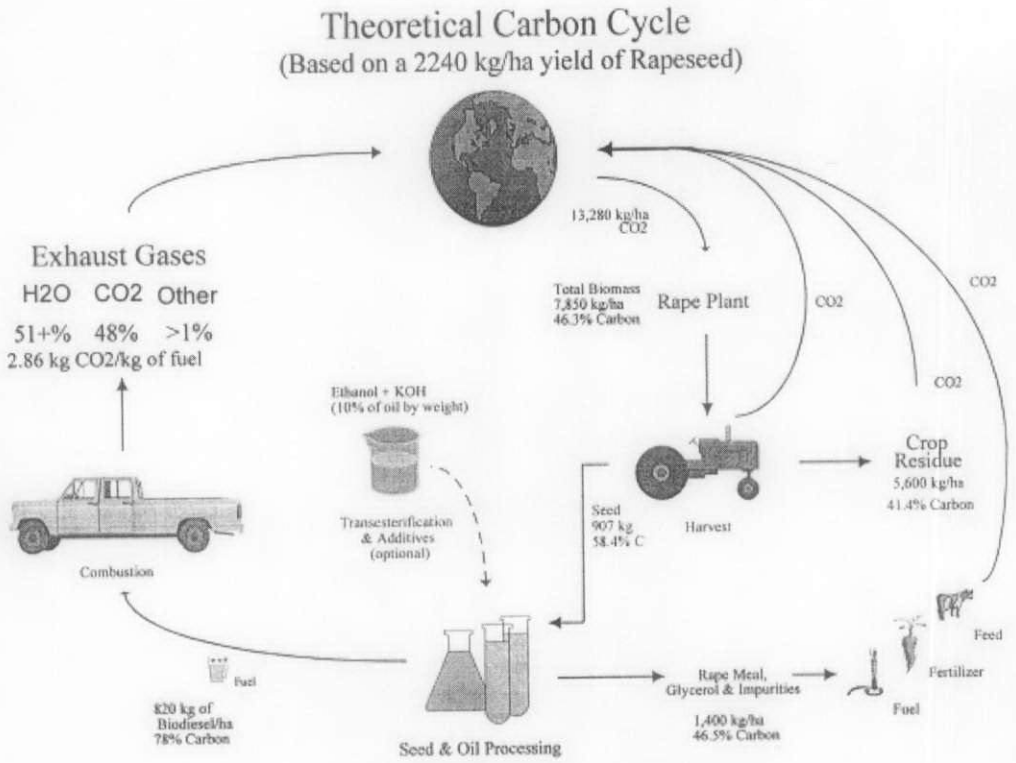


Fig. 3. The rapeseed oil carbon cycle.

Figure 2.3.1: Theoretical Carbon Cycle on Rapeseed Oil[4]

Biodiesel production requires a transesterification process which reacting the rapeseed oil with the alcohol (ethanol/methanol). This produces esters of fatty acids, and glycerol as its by-product. The advantage of transesterification is that it produces biodiesel with a higher cetane rating and lower viscosity than the vegetable oil. The addition of alcohol to the process also brings with it additional carbon but the separation of glycerol during transesterification removes carbon from the mixture. Therefore, it is estimated that

biodiesel releases 1.1 to 1.2 times the CO_2 released from petroleum diesel. This CO_2 from biodiesel however will be recycled by a future rapeseed plant.

The problem with biodiesel production is the high cost. Currently the production cost for fossil-fuel diesel is 17p/litre, but for biodiesel is 34p/litre (using rapeseed oil)[5]. This is much higher than the usual biodiesel price produced in massive capacity. This is why there is a need to combine extraction and reaction stages for a reduction in running and capital cost of production, which would encourage interest by small and medium enterprises.

2.3 BIODIESEL PROCESSING

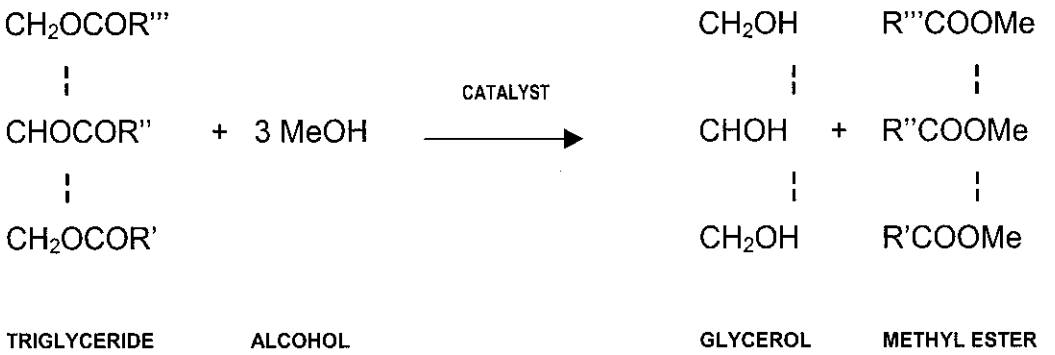
Biodiesel, can be produced from three basic routes via oil and fats: base catalysed transesterification, direct acid catalysed of the oil with methanol, and conversion of the oil to fatty acids, and then to alkyl esters with acid catalysis.

The most favoured route is via transesterification, where alcohols such as methanol and ethanol are reacted with the vegetable oil, in the presence of liquid catalyst such as potassium methoxide (KOMe) and sodium methoxide (NaOMe) to produce methyl ester or ethyl ester. Transesterification is the most economic for several reasons[6]:

- Low operating temperature and pressure
- High conversion with minimal side reaction and reaction time
- Direct conversion to alkyl ester with no intermediate steps
- Exotic materials of construction are not necessary

2.3.1 Kinetics of Transesterification

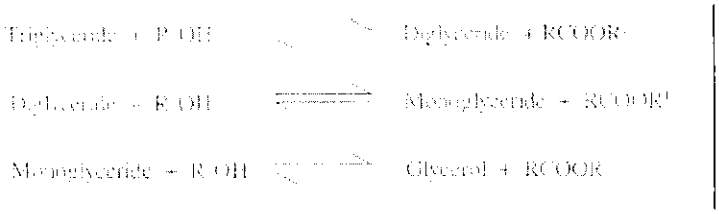
The stoichiometric equation of transesterification reaction is shown as below:



Equation 1

As can be seen, the molar ratio on alcohol presumably ethanol or methanol to tryglycerides of vegetable oil should be 3:1. However, this is not necessarily true in practice since there are also side reactions.

Generally, in a transesterification reaction, triglycerides produce fatty acid alkyl esters and glycerol. The glycerol layer will settle down at the bottom of the reaction vessel. Diglycerides and monoglycerides are the intermediates in this process.



Equation 2

The step-wise reactions are reversible. Therefore, excess alcohol is required to shift the equilibrium towards the formation of esters. As the alcohol is in excess, the forward reaction is pseudo-first order and the reverse reaction is found to be second order[7]. The rate constant of the reaction can be determined, based on the increased amount of product that occurs in a certain reaction time interval or based on decreased amount of one reactant, which in this context is vegetable oil.[8]

As the alcohol is in excess, the remaining alcohol can be recycled. The glycerol, as the by-product, needs to be separated from the biodiesel itself. After the transesterification stage, the product and the by-product, the catalyst and unreacted reactants needs to undergo neutralisation and washing to remove unwanted substances.

2.3.1.1 Kinetics in Supercritical Fluids

There are at least two problems associated with the presence of catalyst in biodiesel production; the process is relatively time-consuming and purification of the product is necessary.

To shorten the residence time of production, simple ethers such as tetrahydrofuran can make the rapeseed oil and methanol, mixed and became one phase and the biodiesel can be produced in less than 15 minutes depending on catalyst concentration. However, this would not eliminate the catalyst problem.

The development of a supercritical fluid based process would remove both problems as mentioned earlier. Kusdiana and Saka[9] discussed the kinetics of transesterification of rapeseed oil to biodiesel fuel, where the methanol is in a supercritical state, without the presence of catalyst.

Purification of methyl esters from catalyst can be avoided as Kusdiana suggests by the uncatalysed transesterification of vegetable oil in supercritical methanol. The supercritical methanol is believed to make the oil/methanol mixture single phase, due to a decrease in the dielectric constant of methanol in a supercritical state. The reaction is found to be completed within 2 to 4 minutes. The separation process hence becomes simpler and environmentally friendly.

During the process, the entire reaction vessel was immersed in a tin bath, which was preheated at designated reaction temperatures and kept for a set time interval for subcritical and supercritical treatments of methanol (200-500°C). For reaction temperatures below critical temperature of methanol (<239°C), an oil bath was used with a temperature controller.

From the results and the analysis, it was found that the molar ratio of methanol to rapeseed oil is one of the most important variables affecting the yield of methyl ester. At higher molar ratios the methyl esters increase, with a commensurate decrease in the intermediate compounds, such as monoglycerides and diglycerides. The optimum was found to be 42:1. This differs from the ratio required in conventional biodiesel production with the presence of catalyst, where increasing the molar ratio of methanol to oil above 5.67 would make the separation of biodiesel and glycerol difficult

As the operating temperature was varied from 200°C to 500°C, it was found that the conversion increased significantly from 68% (200°C) to 95% (350°C). An important result was that the composition of methyl esters yielded was very similar to that prepared by the conventional commercial process with alkaline catalyst. At 400°C, the rapeseed oil is completely converted into methyl ester but at the same time, thermal degradation occurred, which decomposed the product. Hence, the optimal condition was 350°C.

Another important result from this experiment was that no intermediates were formed, so the final products of the reaction in supercritical methanol are methyl esters and glycerol only, and therefore simpler mathematical model for this reaction can be derived. The reaction is assumed to proceed in the first order reaction as a function of the concentration of triglycerides and reaction temperature.

In Madras *et al*[10], three types of supercritical fluids were used in the transesterification reaction: supercritical methanol, ethanol and CO_2.

The experiment was conducted using sunflower oil with supercritical methanol and ethanol without the presence on any catalyst. However the synthesis of biodiesel using supercritical CO_2 was performed using enzyme catalysis. A constant molar ratio of alcohol to oil of 40:1 was used for this experiment whilst, for the supercritical CO2 experiment, a molar ratio of alcohol to oil of 5:1 with an enzyme loading of 30 wt% of oil was used.

The temperatures used in the synthesis for both methanol and ethanol were varied in the range of 200 – 400 °C, where the critical temperatures of methanol and ethanol are

240°C and 243°C respectively. It was found that as the temperature increases, the yield increased from 78% to 96%. Supercritical ethanol gave a higher conversion than supercritical methanol. This may be attributed to the solubility of the oil in ethanol since the solubility of ethanol is lower than that of methanol and it is much closer to the solubility of the oil.

The variation of temperature in this experiment allowed Madras to determine the activation energies for both supercritical fluids in uncatalysed transesterification processes. It was found that supercritical ethanol and methanol had activation energies of 2.0 kJ/mol and 3.0 kJ/mol respectively.

Biodiesel synthesis using supercritical carbon dioxide gave undesirable results. For vigorous stirring between 1-12 hours, the conversions to methyl and ethyl biodiesel at the optimum conditions were 23% and 27% respectively. Greater enzyme loading of over 30wt% with respect to sunflower oil did not significantly increase the conversion. This method however is unfavourable due to the prohibitive cost of enzyme.

Biodiesel synthesis using supercritical alcohols offers a potentially low cost method with simpler technology. However, supercritical processes are much more energy intensive than the conventional alkali-catalyzed process and any feasibility study will have to examine the economics for both conventional and supercritical processes.

2.3.2 Choice of Catalyst in Transesterification

Transesterification, also known as alcoholysis can be catalysed by both homogeneous and heterogeneous catalysts. Further details are described in below:

2.3.2.1 Homogeneous Base Catalyst

The homogeneous catalysts include alkali and acids type of catalysts. The most commonly used catalysts are potassium hydroxide, sodium hydroxide and sodium methoxide. Acid catalysts include sulphuric acid, hydrochloric acid and sulfonic acid. However, these acid catalysts have been studied less.

According to Aracil *et al*[11], the most common catalysts used are base catalysts since the process is faster and the reaction conditions are milder. However, their usage normally produces soaps as undesirable side products. These soaps partially consume the catalyst, decrease the biodiesel yield and complicate the separation and purification steps.

The base catalysts that normally produce soaps are from sodium hydroxide or potassium hydroxide, since they contain the necessary hydroxide group (OH). However, methoxide group does not produce soap through triglyceride saponification as basic methoxides contained hydroxide only as an impurity, which in small quantity and therefore did not produce soap.

Aracil described a comparison of different basic catalysts: sodium methoxide, potassium methoxide, sodium hydroxide and potassium hydroxide for transesterification of sunflower oil. It was found that potassium methoxide and sodium methoxide gave higher yields of biodiesel compared to the other two basic catalysts. As for biodiesel purity, all of the catalysts approached 100% purity after 3 hour of reaction. Saponification was the most significant when using sodium hydroxide and then followed by potassium hydroxide. It was found that there were very small amounts when using methoxide catalysts. Sodium hydroxide had higher contents of methyl ester in glycerol, again followed by potassium hydroxide. Analysis of these two side products indicated that sodium hydroxide is the worst catalyst among these four. Ultimately, using sodium hydroxide would convert the triglyceride faster into methyl esters compared to other catalysts.

2.3.2.2 Heterogeneous Base Catalyst

Conventional biodiesel processing methods, involving homogeneous catalysts such as potassium or sodium hydroxide, produce large amounts of waste water used to separate and clean the catalyst and the products. For an environmentally benign process and the reduction of the production cost, a new process using heterogeneous catalysts should be introduced. Heterogeneous catalysts include enzymes, titanium-silicates, alkaline-earth metal compounds, anion exchange resins and guanadines heterogenized on organic polymers[12].

The heterogeneous catalyst chosen for experiment by Kim *et al*[13] was a base catalyst, Na/NaOH/γ-Al_2O_3. This catalyst was prepared by successive treatment of γ-Al_2O_3 which has been pre-treated at 550°C for 12 hours to remove chemical species adsorbed on surface and further treated with NaOH and sodium at 320°C in the presence of nitrogen.

Four main procedures were used to characterize the catalyst used. The first one was BET Surface Area to measure surface area, pore volume and pore diameter for different combinations of Na, NaOH and γ-Al_2O_3. γ-Al_2O_3 component has the largest BET surface area and pore volume but Na/NaOH/γ-Al_2O_3 has the largest pore diameter. XPS testing was conducted to investigate the binding energy and recording it through a spectrometry with specific electron voltage and resolution. It was found that Na/NaOH/γ-Al_2O_3 binding energy was intensified and indicated a formation of a stronger site. XRD analysis was tried, but no information on the state of sodium could be obtained. From the temperature programmed desorption (TPD) analysis, Na/NaOH/γ-Al_2O_3 had the strongest base sites, which explained well XPS and XRD analysis.

Homogeneous catalyst systems in optimal condition converted triglycerides to biodiesel 20% higher than the heterogeneous system. During the reaction of heterogeneous catalyst, it was found that the reactants were separated into two phases due to the lack of NaOH, which retarded the reaction rate. To overcome this problem, an appropriate co-solvent was used, which in this case was 10 ml of n-hexane in 50 ml of soy bean. The yield increased by 10%. Stirring speed had no effect on production yield.

According to the stoichiometric reaction, the molar ratio of methanol to triglyceride is 3:1. However, it was found that the optimal conditions for this reaction occurred at a molar ratio of 9:1. This was to ensure that the reaction proceeds smoothly.

Na/NaOH/γ-Al_2O_3 was found to be a good heterogeneous catalyst for transesterification of biodiesel, since it has strong basic sites for reaction taking place. Apart from that, the usage of co-solvent such as n-hexane well suited the intended project i.e. combined extraction and reaction of vegetable oil, as commonly vegetable oil extraction uses hexane as solvent. Therefore, there is a probability of using this type of catalyst in further experiment for extraction and reaction of vegetable oil.

2.4 VEGETABLE OIL EXTRACTION

Typical vegetable oil extraction is divided into two types: mechanical press and solvent processing[14]. The mechanical press is carried out by using cold or hot pressing. The feed is heating up the feedstock at 110°F (~317 K) for cold pressing or up to 383-393 K for hot pressing. The feedstock (in our case rapeseed) is crushed in a screw press. The cold pressing method requires less energy for processing of 1 tonne of seeds and there is less phospholipid in the oil, which is desirable for biodiesel production. However, 12-14% of oil is left in the cake, which is an unacceptable loss of yield[15]. This remaining seed meal is used as animal feed.

Solvent processing also known as solid-liquid extraction can extract more oil than mechanical press processing. This process uses solvent to dissolve oil and a further distillation process separates oil from the solvent. The solvent is further condensed, recycled and reused in the process. Vegetable oil is produced with a higher degree of purity than by the mechanical press method. Solvent processing resulted in only 0.1-0.8 % of oil being left in the cake. However, the equipment used is more expensive and the quantity of phospholipids in the hexane-extracted oil is twice as high as that in the pressed oil. For this reason, additional energy consumption is expected for oil degumming processes before transesterification of biodiesel oil can proceed[16].

Rosenthal, Pyle and Niranjan[17] discussed the crop structure of oil-bearing materials. The walls surrounding the seed's cell are primarily composed of cellulose, hemicellulose and lignin in addition to pectin. In the usual solvent-based process, the grain is flaked, thereby causing the seed's cell to rupture and exposes the oil located inside the cell, further facilitating the percolation of solvent into which the oil can diffuse.

2.4.1 Choice of Solvent for Liquid Extraction

Conventionally, modern solvent-based extraction consists of successive extraction by countercurrent washes with hexane of the previously cracked, flaked, ground or pressed oil-bearing materials. Hexane is then removed from the oil in rising film evaporators followed by vacuum distillation. This typical process is depicted as in Figure 5.1[18]

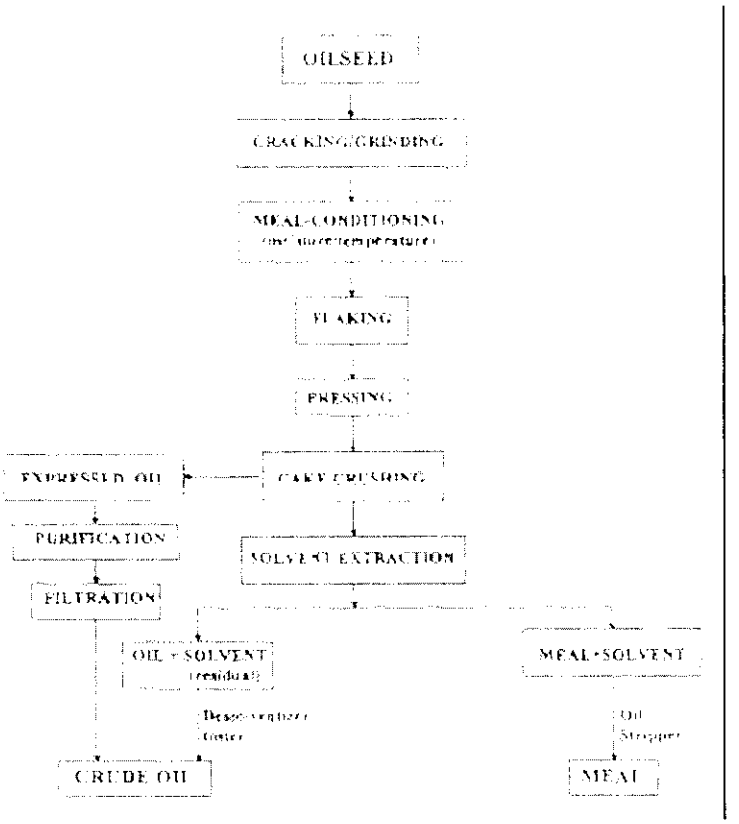


Figure 2.4.1: Conventional Oil Extraction Process

As hexane is removed, the crude oil is submitted to the refining process to remove oil-soluble and oil-insoluble impurities. This involves degumming to remove phospholipids. Free fatty acids, colour bodies, and metallic pro-oxidants are removed to a varying degree in alkali-refining step, which improves the oil taste. Remaining off-flavours are removed by high-vacuum steam distillation in the deodorization step.

Aqueous and enzymatic processes may be attractive for edible oil extraction alone to ensure high quality of recovered oil. However, it is expected that in-situ alcoholysis would not favour this process as enzymatic oil recovery might be expensive. Furthermore, it is not wise to allow all the aqueous content of the oil extraction into the biodiesel synthesis, as this would favour saponification in the reactor.

Niranjan *et al*[19] expressed great concern over the usage of hexane as a solvent in edible oil extraction for environmental reasons. Conventional processes using hexane as solvent would result in hexane loss and associated pollution problems. Hexane can

react with atmospheric pollutants such as nitrogen oxide in the presence of the sunlight to form ozone and other species collectively known as *photochemical oxidants*.

From the safety point of view, hexane is flammable, and in spite of elaborate precautions which have been developed to avoid fire and explosions hazards, there is still a finite danger of severe accidents.

Diosady *et al*[20] in his article provides an alternative to edible oil extraction. In this work, Chinese rapeseed oil is extracted using three-phase extraction in Karr column. Two types of solvents were used: the polar phase consisted of methanol containing 10% ammonia and 5% of water and the non-polar phase was hexane. This is believed to produce meal essentially free from glucosinolates; sulphur-containing compounds which hydrolyse to produce toxic substances, and the most bitter phenolic constituents of the seed. The rapeseed oil was crushed and mixed with the polar phase ($CH_3OH/NH_3/H_2O$) to wash out the glucosinolates, and then passed to hexane for oil extraction and back with this polar phase during washing stages.

This breakthrough is interesting since methanol alone can wash out the glucosinolates and remove other phenolic constituents and produce animal meal at high quality. This implies that if hexane and methanol were blended together, oil extraction and biodiesel synthesis in the presence of alkaline catalyst can be implemented and finally removes these glucosinolates and phenolic constituents for high quality of animal feed.

Another solvent for oil extraction is ethanol. Meirelles *et al*.[21] studied the liquid-liquid equilibrium data for systems of rapeseed oil, oleic acid and short-chain alcohols such as methanol, ethanol, isopropanol and n-propanol. They found that the mutual solubility of oil and solvent increased with increasing carbon chain length of the solvent and the temperature. The coefficient of distribution term was introduced as defined below:

$$K_i = \frac{C_i^{II}}{C_i^{I}}$$

Equation 3

In the experiment, methanol had a coefficient of distribution less than 1, whilst ethanol's was greater than 1. This would result in ethanol having greater extraction capacity

compared to methanol, where ethanol dissolves more rapeseed oil, and less selectivity of fatty acids in oil extraction than methanol.

Another study, carried out by Meirelles *et al*[22] on equilibrium data for corn oil oleic acid and ethanol, but in the presence of water. The distribution coefficient of fatty acid is reduced when ethanol is mixed with water indicating that aqueous ethanol has a lower extraction capacity for fatty acids. Otherwise, the addition of water increases the solvent selectivity and consequently reduces the loss of neutral oil in solvent extraction. Meirelles finally concluded that additional water content between 4 and 6 wt% in the aqueous ethanol is appropriate for deacidification by solvent extraction as it still provides values of fatty acid distribution coefficient around unity, and high values for the solvent selectivity.

Various studies have also been carried out on supercritical extraction especially supercritical carbon dioxide (Sc-CO_2). Sovova *et al*[23] investigated on sea buckthorn oil extraction with supercritical CO_2. The product is natural, free from solvent and has not undergone thermal degradation, since the extraction can be carried out at low temperature.

The extraction of sea buckthorn oil in the study used supercritical CO_2 at temperatures in the range 25-60°C and operating pressures between 9.6 MPa and 27 MPa. Almost 100% of the triglycerides were extracted using this solvent.

2.5 COMBINATION OF EXTRACTION AND REACTION OF VEGETABLE OIL – A NEW CONCEPT

Alcoholysis or transesterification, of vegetable oils would benefit from combination of extraction and reaction processes, as this would lead to potential savings in money, time and energy. There are very few studies that have been carried out regarding the combination of both extraction and reaction of vegetable oil. However, the articles that will be described in this chapter provide a broad overview of simultaneous extraction and reaction of vegetable. The differences between conventional and in-situ transesterification are shown in Figure 6.1 below:

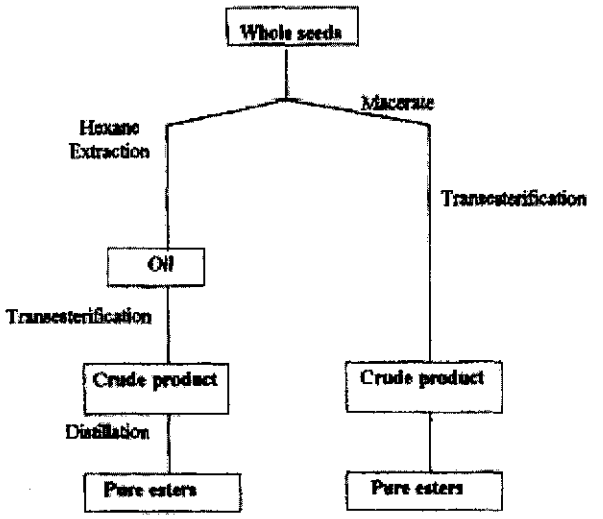


Figure 2.5.1: Difference Between Conventional (Left) and In-Situ Transesterification (Right)

2.5.1 In-Situ Transesterification – An Overview

Türkay et. al. discussed in-situ alcoholysis of soybean oil. The idea of in-situ alcoholysis is that the oil-bearing material contacts directly with acidified alcohol instead of reacting purified oil and alcohol, indicating that alcohol acts as both extraction solvent and esterification reagent.

The experiment was carried out using various short chain alcohols, such as methanol, ethanol, n-propanol and n-butanol. The catalyst used was sulphuric acid. Prior to the

reaction, the soybean seed was ground and added with alcohol containing concentrated sulphuric acid. The mixture was agitated and the filter cake was washed with alcohol. This filter cake was dried overnight at room temperature and re-extracted using hexane to obtain the remaining oil. Ratio of oil between the first reacted in-situ and the second one with hexane then was calculated. Two types of pre-treatment were used: conditioning or maceration. Conditioning was performed by heating the ground beans first, whilst the ground beans were soaked in methanol for macerating.

It was found that in most of the alcohols, in-situ alcoholysis dissolved more oil than a single extraction. Macerating the seed caused the cell walls of the soybeans to break down, leaving the oil free and available to react with the alcohol, resulting in higher oil extraction rate.

In a single extraction by 96% ethanol, increasing extraction time did not affect the amount of oil extracted, but in-situ alcoholysis increased the amount of oil extracted, by increasing the reaction time. This suggested that, as triglycerides dissolved in ethanol were converted to ethyl esters, the concentration of triglycerides in oil phase would be reduced would result in further extraction. The overall reaction rate was determined by the extraction and alcoholysis rates.

Another experiment, where ground particle size was the variable indicated that smaller particles during in-situ alcoholysis would result in higher extraction and reaction rate. However, in single extraction, the change was insignificant. This implied that the surface area of the particle increased, allowing further extraction to take place. Washing the filter cake after in-situ alcoholysis with methanol or ethanol was necessary, as this would help to increase the amount of oil dissolved during the reaction, since there was some amount of solution held by the solid after filtration.

Further testing was carried out on extraction and reaction of soybean seeds with the monohydroxy alcohols: methanol, 96% ethanol, 98.8% ethanol, n-propanol and n-butanol. Increasing the alcohol chain length caused the amount of oil dissolved to increase. Increasing the amount of water, as in between 96% ethanol and 98.8% ethanol, resulted in a lower dissolution rate indicating that anhydrous ethanol as well as aqueous are not good solvent for soybean oil at normal temperatures. The free fatty

acid component of the esterified products obtained by the in-situ alcoholysis of soybean oil with monohydroxy alcohols was higher than that of the crude soybean oil in seeds indicating hydrolysis reaction occurred during in-situ alcoholysis.

Türkay concluded that ethyl, propyl and butyl esters of soybean fatty acids could be obtained directly in high yields by in-situ alcoholysis of soybean oil. The variables that will affect the output yield were reaction temperature and time, maceration, washing stage, water content in alcohol and particle size of seeds.

Türkay and Ozgül,[24] also conducted experiments on in-situ esterification of rice bran oil with 99.7% methanol and 96% ethanol. Sulphuric acid was used as the catalyst. Two in-situ esterification reactions were carried out for methanol where one mixture was uncatalysed and the other one was catalysed by sulphuric acid. The uncatalysed mixture did not react at all. It implied that these components did not dissolve in the methanol but remained in the bran. Another investigation carried out was the effect of catalyst on in-situ esterification. It was found that higher quantities of catalyst resulted in faster conversion to alkyl ester. This indicated that the quality and the quantity of the methyl ester fractions were dependent on free fatty acid (FFA) content of the oil. In practice, it was observed that all FFA were dissolved in methanol and esterified, whereas non-soluble triglycerides and other non-polar components of the oil remained in the bran. The solubility of triglycerides in methanol increases with increasing FFA in the medium.

The effect of the FFA content of the oil on esterification was investigated by esterification of bran with different storage histories. It was demonstrated that as the storage days increased, the FFA content of the oil increased, which led to higher percentages of oil being converted to methyl esters.

As for in-situ esterification with ethanol, the same experiments were carried out. No ethyl esters were detected when catalyst was not present but in the catalysed esterification, there were still some unreacted FFA's left behind, even though the quantity of the catalyst was increased. However, when ethanol with different FFA contents was esterified, the ethyl ester produced was not dependent on FFA content of bran since the solubilities of the oil components in ethanol were much higher than those

in methanol. Therefore it was concluded that relative compositions and the amount of esters that can be obtained from a given rice bran were dependent on the solvent itself. Purer esters can be obtained from methanol, as methanol has higher selectivity than ethanol. For low acidity rice bran oils, in-situ esterification with methanol is also known as deacidification, since all FFA are removed from the bran as methyl esters, whereas practically all of the triglycerides remain in the bran.

Siler-Marinkovic and Tomasevic[25] discussed the potential of in-situ transesterification of sunflower oil for sunflower seeds macerated in methanol. The slurry was mixed up with methanol and sulphuric acid for extraction and reaction. Two operating temperature were chosen, 30 °C and 64.5 °C and the reaction time was varied between 1 to 4 hours. The filter cake was further washed and re-extracted with chloroform to yield further esterified product.

The yields of methyl esters obtained from in-situ reactions were greater than those from conventional treatments. As for acid-catalysed methanolysis, a higher molar ratio (up to 45:1) was required for a good conversion. Molar ratio is strongly dependent on other variables too, such as amount of catalyst and reaction time. It was determined that a molar ratio of 300:1, in the presence of acid catalyst of 100 wt% on oil basis and reaction time within an hour would allow full completion of transesterification. However, it was reported that the acid catalyst would make the production slower than alkali catalyst but the biodiesel produced is of lower viscosities. In-situ transesterification would also give a lower cloud point than those prepared by conventional processing.

An interesting online extraction-reaction of rapeseed oil with ethanol by immobilized lipase in supercritical CO_2 was developed by Goto *et al*[26]. The operating temperature was varied between 308 K (35°C) and 328 K (55°C), whilst the operating pressure was varied between 24 MPa and 35 MPa. Fatty acid ethyl esters were synthesized from extracted rapeseed (canola) oil with ethanol using immobilized lipase.

Initially, the canola flakes were loaded into the extraction cell. Then the system was pressurized and the system temperature was built up to reach desired value. When both temperature and pressure reached the desired state, the ethanol was introduced and depressurization took place whilst trying to maintain the flow of CO_2 at ambient

condition. Reaction product samples were collected between 20 and 120 minutes along the reaction.

The studied parameters were ethanol quantity, enzyme loading, temperature and pressure. Ethanol quantity was varied by varying the ethanol flow rates, corresponding to about 1.0, 5.0 and 10.0 wt% of CO_2 mass at standard conditions. It was found that at a temperature of 308 K, and enzyme loading of 1.0 g, the ethyl esters produced were reduced as the amount of ethanol increased to 10.0 wt%. This suggested that excess ethanol inhibited the enzymatic reaction as the enzyme activity is highly sensitive to a change in pH and the concentration of the entrainer e.g. methanol or surfactants. However, the higher concentration of ethanol tends to increase the amount of diglycerides and free fatty acid (FFA) mainly due to the change in the solubility of the material in Sc-CO_2. This indicated that a low degree of reaction took place, as amount of ethanol increased.

Enzyme loading was varied by using different amounts of immobilized enzyme at specific temperature and pressure (35MPa and 308K). It was found that a greater extent of ethanolysis of extracted oil was achieved with a larger amount of enzyme in the system. Another important investigation was to study the effect of water content on the enzyme activity by use of anhydrous ethanol and bone-dry CO_2, since water content complicates the process by changing solubility, vapour pressure, etc. and so forth. In this experiment, however, extra water may be needed to make up for the water consumption due to increased enzyme load.

The effect of the operational conditions was investigated by varying the operating temperature (308K and 328K) and pressure (24 MPa and 35 MPa). Interestingly, operating at lower temperature favour extraction, whereas at higher temperatures favour ethanolysis. At fixed temperature, the yield of extracted oil increased with pressure. However, the percentage of ethyl esters produced remained the same and the percentage of diglycerides increased by a factor of two.

This experiment was difficult to optimise for both extraction yield and efficient reaction. There is potential for further development on this process as each unit operation could be carried out at its optimum condition sequentially.

Haas *et al*[27] conducted in-situ alkaline transesterification as an effective method for the production of fatty acid esters from vegetable oils. Design of experiment and response surface regression analysis was adopted to optimise the reaction conditions. By using flaked soybeans of 5 g amount and NaOH dissolved in methanol solution, the transesterification was carried out at two different temperatures: 60°C and 23°C. The other parameters being investigated were the amount of methanol, NaOH concentrations, and reaction times. For qualitative analysis, thin layer chromatography (TLC) was used andhigh performance liquid chromatography (HPLC) was used for quantitative analysis.

At temperature of 60°C, incubating the soy flakes in alkaline solutions of simple alcohols resulted in biodiesel production. However, this was produced at low conversion and low yield. Transesterification at this temperature; by varying the methanol amount from 12 ml to 25 ml and NaOH concentration from 0.1 to 0.2 N yielded in high methyl esters with low contamination of free fatty acid (FFA) and triglycerides. The reaction time was approximately 6 hours. Haas also inferred that at higher amount of alcohol, smaller amount of catalysts were required for good yields and low contamination. He concluded that in-situ method required about 38 times more alcohol and 7 times more alkali than a conventional method did.

At temperature of 23°C, the transesterification also occurred but for an optimal condition to be achieved, 90 times more methanol and 9 times more NaOH were required. This process offers the advantage of efficient operation using soy flakes prepared by current industrial technology, less reagents used, and substantially higher ester yields. Haas finally concluded that direct in-situ transesterification is useful where smaller scale operations can generate a marketable product from the oil portion of an oilseed crop.

The oil-free meal as an animal feed was another factor that should be taken into account since this is an essential component to the economic viability of an oilseed-processing operation. Economic viability of the method described here depend on whether the extracted flakes are nutritionally and organoleptically suitable for use as animal feed.

2.6 LIQUID MEMBRANE

Liquid membrane is a type of membrane, which is made of liquid. Liquid membranes are highly selective and with the use of carriers for the transport mechanism, specific molecular recognition can be achieved. Liquid membranes are relatively high in efficiency and as such are being looked into for industrial applications. Liquid membranes require stability in order to be effective and if they are pushed out of the pores or ruptured in some way due to pressure differentials or turbulence, then they just do not work[28].

There are a few types of liquid membranes: bulk liquid membranes, emulsion liquid membranes, thin sheet supported liquid membranes, hollow fibre supported liquid membranes and two hollow fibre supported liquid membranes and some more. Emulsion liquid membrane (ELM) is a type of liquid membrane which has a very thin membrane and a large surface area per unit source phase volume. This would enhance the transport rate of this membrane. Concentrations in the receiving phase are increased by a large factor, due to the ratio of source phase volume to receiving phase volume, which occurs whenever the organic phase emulsion is added to an even larger quantity of source phase.

For stability, all that is required is for both the membrane solvent and the carrier molecule to be mildly hydrophobic. Compared to the hollow fibre system, the volume ratio is not large since the organic and receiving phase volumes are equal and large source volumes cannot be used if it is intended to maintain that large area per source phase volume ratio mentioned earlier. Typical ELM is shown as follows[29]:

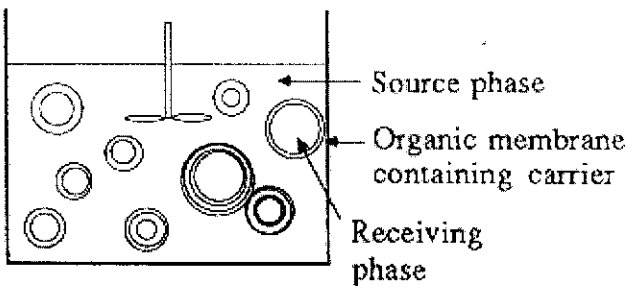


Figure 2.6.1: Typical Emulsions Liquid Membrane

Washington[30] in his review discussed on the stability of emulsions or colloids where the stability depends on the forces between them; if these are repulsive, approaching droplets repel and form a stable system. If they are attractive, the droplets clump together and are unstable. The forces can arise from Van der Waals forces, electrostatic forces, solvent forces and steric forces. In general, colloids are stabilized either by steric or electrostatic repulsion, depending on the nature of the surfactant. Note that emulsions between two liquids are formed through the addition of surfactants or stabilisers. Stability of water in oil emulsions as reported by Tao and Chen[31], is defined as the resistance by the dispersed water droplets against coalescence and dependent on a variety of factors: presence or absence of emulsifying agent, viscosity (influenced greatly by temperature), specific gravity, water content and the age of emulsion.

Tao and Chen further described the concept of hydrophile-lipophile balance (HLB) where the molecule of any emulsifier, or indeed of any surface active material, contains both hydrophobic and hydrophilic groups and the ratio of their respective weight percentages should influence emulsification behaviour. Water in Oil (W/O) emulsifier will have low HLB, a solubilizing agent has a high HLB and and Oil in Water (O/W) emulsifier has an intermediate value. It is important to know that the emulsification is best when the emulsifier and the emulsified material have the same HLB value. Torres *et al*[32] showed that HLB is an empirical parameter that describes the relative contribution of the hydrophilic moiety to the weight of the surfactant molecule. Surfactants with an HLB number of 3-6 are lipophilic and can be used for water in oil emulsions. Higher HLB values in the range of 10-18 are more hydrophilic and can be used to prepare oil in water emulsions.

Surfactant dosage is the most important factors influencing the stability of emulsions. The emulsification is stabilised up to a certain limit when increasing the dosage. As the concentration is increased further, emulsion stability will decrease. At low surfactant concentrations, the emulsion is not stable due to agglomeration of the oil droplets; at high concentrations, the emulsion destabilization occurs as a result of rapid coalescence.

Oil to water ratio in the case of emulsification of water in oil is another main factor that affect the stability too. Tao and Chen reported that when the ratio of oil and water were almost the same, more stable emulsion can be created. Stirring intensity also influenced the stability of emulsions where the purpose of stirring is to form a stable and homogeneous emulsion by breaking large liquid droplets into smaller one. The higher the stirring intensity, the better the stability obtained. In this case it was reported that the highest stirring rate applied was 3000 rpm. However this is not required since higher stirring speed would also lead the emulsifier to break away from the emulsified material interface.

Mixing temperature did affect the stability of emulsions as well. Obviously, emulsion was more stable at low temperature since the surface tension of most liquids decreases with increasing temperature. An abnormal increase of temperature shall be avoided because it tends to coagulate the particles, thereby causing a deterioration of the emulsions. Apart from that the interfacial adsorption of the emulsifier is adversely affected to some extend by increasing temperature and the surfactant will separate out from the emulsion; resulting in collision and coalescence. This would definitely destabilise water in oil emulsions.

Finally, the mixing time is another important factor for emulsification. Radii of emulsification droplets will decrease with increasing of mixing time. Therefore the emulsifier becomes more effective. However if the mixing time is too long, the effectiveness of emulsifier dropped out from the oil-water interface. Results showed that the best mixing time was between 10 to 15 minutes and further increased will lead to destabilisation of the emulsions.

2.7 BIODIESEL AND EXTRACTED OIL ANALYSIS TECHNIQUES

One of the most important stages of this experimental process is analysis, in this case to determine the quality of oil extracted and the yield of biodiesel produced.

The main parameters governing quality of oils extracted are the amounts of oil extracted, amount of triglycerides, acid number, peroxide value, saponification value, iodine value and phospholipids.

In analysing the biodiesel quality, the parameters can be divided into two groups[33]. One group contains general parameters, which are also used for mineral oil based fuels, and the other group specifically describes the chemical compositions and purity of fatty acid alkyl esters. In the first group, the main parameters are the viscosity, density, flash point, pour point, neutralization number, pH, cetane number, and cold filter pour point (CFPP).

The second group consists of methanol/ethanol content, ester content, monoglycerides, digylcerides, triglycerides, free glycerol, total glycerol and iodine number.

2.7.1 Oil Extraction Analysis

The amount of oil extracted in in-situ transesterification can normally be measured by vacuum distillation or vacuum filtered technique in a Buchner funnel,[34] where all the extracted oil is separated from the oil-bearing materials and the solvent. Later, to determine the residual oil in the oil-bearing material, the filter cake can be washed with alcohol, left overnight for drying and the residue re-extracted in a Soxhlet apparatus with hexane or chloroform to obtain the oil in the cake. The ratio of the residual oil to the total amount of the oil in the seed can be calculated to give the percentage of oil dissolved in the solvent used earlier (in this case alcohol).

The acid number is used for characterizing oils and fats and analysing the degree of purity of free fatty acids[35]. It is also used to determine the concentration (content) of free (fatty) acids in oily substances (lipids). The acid number is determined from a simple

equation. Initially, the sample to be determined is weighed and further titration takes place between ethanol and potassium hydroxide (KOH). Neutralised ethanol is mixed with the sample and further titration performed with KOH. The amount of KOH consumed to neutralize the mixture of neutralised ethanol and sample can be noted and measured using this equation:

$$AN = \frac{a * 28{,}0505}{E} \quad \textbf{Equation 4}$$

where AN is the acid number, a is the amount of KOH consumed and E is the weight of the sample.

Peroxide value is a method of assessing the hydroperoxide. Oxidation is the most common cause of rancidity in oils and fats, which finally leads to oil deterioration. It is generally accepted that the first product formed by oxidation of oil or fat is a hydroperoxide. The most common analytical methods are those based on an iodometric titration, which measures the iodine produced from potassium iodide by the peroxides present in the oil. The peroxide value is expressed as milli-equivalents of oxygen per kilogram of fat or as millimoles of active oxygen per 2kg of the sample[36].

The saponification value can be determined by measuring the amount of excess potassium hydroxide remaining after the saponification of oil or fat. It is a measure of the mean molecular weight of the component glycerides, or fatty acids which is related to the molar mass of the oil or fat, and is defined as the number of milligrams of potassium hydroxide required to saponify 1g of oil or fat, i.e. to neutralize the free fatty acids and the fatty acids combined as acylglycerols. The test is performed by boiling the oil sample under reflux with an excess of ethanolic potassium hydroxide solution. The excess is then back-titrated with standard hydrochloric acid solution using phenolphthalein as indicator. The saponification value can also be determined by calculation, using the fatty acid composition of the sample determined by gas chromatography. This calculation may be applied to all oils and fats, provided they contain only low concentrations of free fatty acids, monoglycerides and diglycerides[37].

The iodine value, as the amount of halogen in grams, expressed as iodine per 100 g sample, indicates the degree and the amount of unsaturation under specific conditions. The sample is dissolved in carbon tetrachloride or chloroform and is allowed to react

with excess of iodine solution for a specified time at ambient temperature in the dark. The excess iodine solution then converts the iodine solution into free iodine by means of an oxidation process. This content of liberated iodine is determined by titration with a thiosulphate solution[38].

Another interesting parameter to measure the quality of oil extracted is the amount of phospholipids in the oil. Phospholipids are phosphorus-containing compounds in oil. They are undesirable as they lead to oil discolouration which necessitates deodorization and steam distillation. They affect the stability of the oil by chelating metal ions, thereby increasing rate of oxidation process. Excess amounts of phosphorous compounds in the fuel can cause damage to the catalytic converter in the diesel engine. A rapid reliable method was developed to measure vegetable oil phospholipids content by thin-layer chromatography-imaging densitometry. Phospholipids concentration can be determined by water degumming and then analysing by thin-layer chromatography and imaging densitometry. The concept of chromatography will be discussed in the next section as to measure free fatty acid content.

2.7.2 Biodiesel Quality Analysis[39,40]

As described before, there are two groups of characterisation parameters for biodiesel. The viscosity controls the characteristic of the injection from the diesel injector. If the viscosity of fatty acid methyl esters became very high levels it would have an adverse impact on fuel injector system performance. Therefore the viscosity specifications should be within the range of diesel viscosity. There are many methods for measuring the viscosity of biodiesel or vegetable oil. Among the most common used are the Ubbelohde viscometer, the Hoppler set, and Engler viscosimeter.

The density is measured by simply weighing the sample and measuring the volume of the weighed sample. Normally, the density of biodiesel would be between 850 and 900 kg/m^3. The flash point of a fuel is the temperature at which ignition will occur when the oil is exposed to a flame or spark. Flash point is measured by heating the sample at a slow, constant rate with continual stirring. A small flame is directed into the cup at regular intervals with simultaneous interruption of stirring. The flashpoint is the lowest

temperature at which application of the test flame causes the vapour above the sample to ignite.

Cold filter plugging point (CFPP) is a measure of the fuel's cold weather performance. It determines the lowest temperature operability of automotive diesel fuel, heating oils and gas oils, including those containing flow improving additives. The fuel sample is cooled and, at intervals of 1 °C; a vacuum of 200 mm water gauge is applied to draw the fuel through a fine wire mesh filter. As the fuel cools below its cloud point, increasing amounts of wax crystals are formed. The fuel thickens which would adversely affect its flow through fuel lines, fuel pumps and injectors. This causes the flow rate to decrease and eventually complete plugging of the filter occurs. Instead of measuring CFPP, another parameter available is pour point. This is the measurement of the lowest temperature at which the oil specimen can still flow. Normally, the lowest acceptable CFPP or pour point value of a biodiesel is approximately -10 °C.

Cetane number indicates the fuel's ignition characteristics. It measures the ease of ignition and the smoothness of combustion. Higher cetane number is due to better ignition properties. Most European countries specified 49 as the minimum cetane number for a biodiesel standard. Cetane number measurement is carried out in a diesel engine in which the exact time of injection and beginning of combustion can be determined. The longer the ignition delay, the worst the cetane number is. Cetane number would affect engine performance parameters such as combustion efficiency, stability, driveability, white smoke, noise and emission of CO and other HC's. Normally, biodiesel would give higher cetane number than conventional diesel fuel, which results in higher combustion efficiency.

Neutralization number is measured to ensure proper ageing properties of the fuel. This measurement reflects the presence of the biodiesel and the degradation of biodiesel due to thermal effects. The lower the neutralization number, the better the performance of the biodiesel. In European countries, the specification is that the neutralization number is below 0.5. This is measured by two methods: by colour indicator titration and potentiometric titration. The first method is performed by dissolving the fuel in a mixture of toluene and isopropyl alcohol containing a small amount of water, and the resulting single-phase solution is titrated at room temperature with standard alcoholic base or alcoholic acid solution. The latter method is performed by dissolving the fuel in a mixture

of toluene and 2-propanol containing a small amount of water and titrated potentiometrically with alcoholic potassium hydroxide or HCl solution, using a glass indicating electrode and a calomel reference electrode.

The second group of parameters described earlier focused on vegetable oil specific parameters. Gas chromatography or high performance liquid chromatography can be used to determine the content of glycerides, free glycerol, unreacted free fatty acid and the alkyl esters produced. Trimethylsilylation of glycerol, mono- and di- glycerides followed by gas chromatography (GC) using a 10 m capillary coated with a 0.1 μm film of DB-5 would allow the determination of all free fatty acid compounds in a single GC run. Trimethylsilylation ensures excellent peak shapes, good recoveries and low detection limits, which improve the robustness of the procedure. Silylation is the most widely used derivatization procedure for sample analysis by GC. The popularity of silylation reagents is due to their ease of use and formation of derivatives[41]. For complete silylation of glycerol and partial glycerides, the conditions of derivatization reaction have to be controlled carefully. Many reports on GC use for biodiesel analysis employ flame-ionization detectors (FID). The sample of peak detection using GC is depicted in Figure 2.7.1[42].

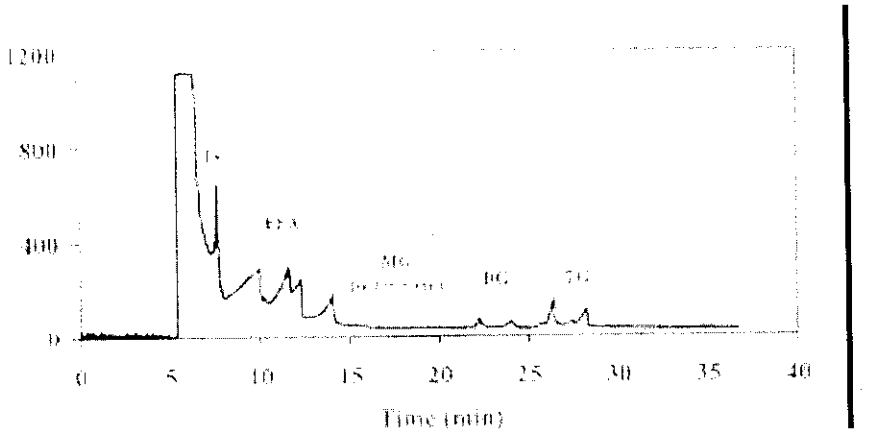


Figure 2.7.1: Sample Results From GC on FFA Measurement

As for high performance liquid chromatography (HPLC), the difference with GC is that time and reagent consuming derivatization are not necessary, so it reduces analysis time. The determination of glyceride content in HPLC can use different detectors such as density detector, amperometric detection, Ultra violet (UV) detection, evaporative light scattering detection (ELSD) and atmospheric pressure chemical ionization mass

spectrometry (APCI-MS)[43]. The density detector and UV detector was appropriate for studying the degree of conversion of the transesterification reaction whilst the amperometric detector is useful for determining the amount of free glycerol in vegetable oil esters. As for rapeseed oil biodiesel, APCI-MS is stated to be the most appropriate detection method for its analysis. A sample HPLC output was depicted in figure 2.7.2[44].

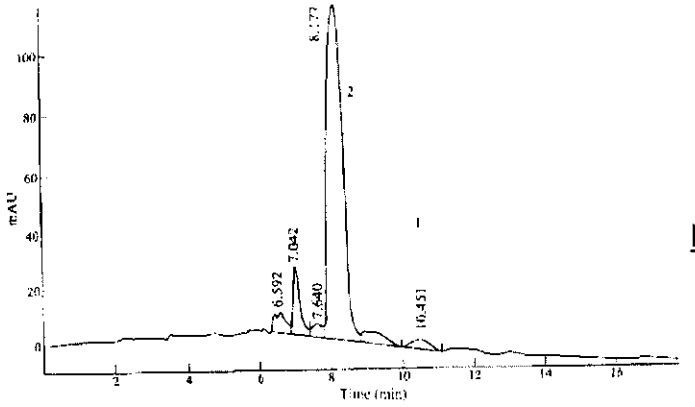


Fig. 1. HPLC chromatogram of buckthorn oil extract (mAU = milliampere unit(s). Identified peaks: (1) free fatty acids, (2) triglycerides.

Figure 2.7.2: Sample Results from HPLC Measurement

2.8 OSCILLATORY FLOW REACTOR – A NEW DESIGN FOR THE NEW CONCEPT?

The oscillatory flow reactor (OFR) is a new type of reactor, consisting of tubes containing equally spaced orifice baffles. These baffles act as stirred tanks and the residence time distribution of this reactor depends upon the interaction of the net and imposed oscillatory flows. An example of OFR is shown as in figure 2.8.1[45]:

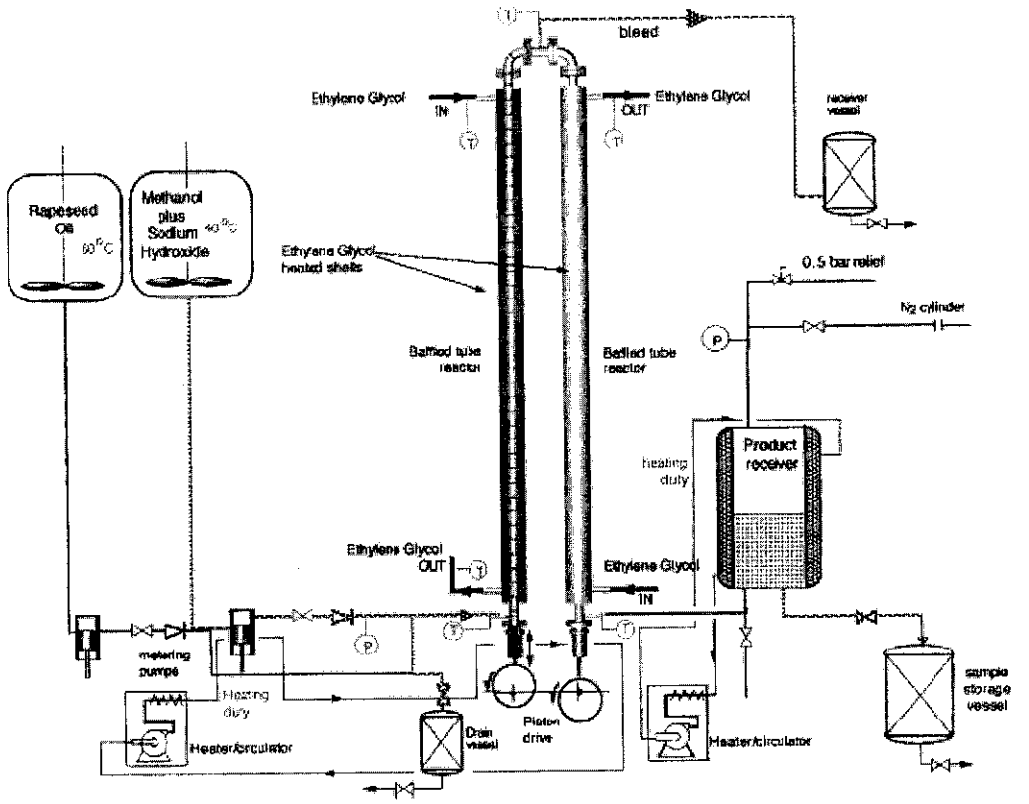


Figure 2.8.1: Oscillatory Flow Reactor developed by Cambridge University

This reactor is characterized based on dimensionless numbers such as net flow Reynolds number (Re_n), oscillatory flow Reynolds number (Re_o), velocity ratio (Ψ), and Strouhal number (Str). A good degree of plug flow can be achieved, based on Ψ ranging from 4 to 10.

The OFR differs from conventional PFR, in its ability to perform long reactions that are usually only practical in batch reactors. Whilst conventional PFR is highly dependent on fluid velocities, the OFR is dependent upon oscillatory conditions and thus can perform many long batch processes in a more efficient continuous manner.

2.8.1 Biodiesel Production in Oscillatory Flow Reactor

Harvey *et al* [46] discussed biodiesel production in the OFR. The OFR used consisted of jacketed by QVF tubes, and had an internal volume of 1.56m^3. The molar ratio between methanol and rapeseed oil was kept constant at 50% excess of methanol, to ensure a high yield. NaOH was the catalyst for this transesterification reaction. The oil feed vessel temperature was maintained at the reactor temperature but the methanol feed vessel operated at slightly lower temperature to avoid evaporation loss. The product was quenched, and allowed to settle. During the experiment, the reactor temperature and residence time were varied whilst others parameter were kept constant.

From the experiment and testing, the quality of biodiesel was determined based on viscosity, density, pH, volume fraction of glycerol, cetane number, glyceride and cold filter pour point (CFPP) analysis. Despite changing the parameters of reactor temperature and residence time, the viscosity, density, pH and volume fraction of glycerol were shown to be consistent and acceptable. The cetane number for all samples increased as residence time and reactor temperature increased and passed the UK standard. However, at 10 minutes residence time and 50°C reactor temperature, too much triglyceride remained unreacted, leaving significant amount of diglycerides, but meeting the German standard for all other glycerides. Another samples contained negligible amounts of triglycerides and diglycerides.

From the samples as well, CFPP ranged acceptably, but in characterizing the reactor, the velocity ratio (Ψ) seems to be too high i.e. 18, which means the reactor was equivalent to eight tanks-in-series. This would still be good for biodiesel production but a reactor of larger L/D ratio should be used to allow Ψ in the desired range. This experiment demonstrated the ability of OFR in biodiesel production but optimisation of the reaction conditions was still on going.

2.8.2 Droplet Size Distribution in Oscillatory Flow Reactor

Studying droplet size distribution is important when combining the extraction and reaction of vegetable oil to biodiesel. This is due to the fact that the reagents to be used

in this particular process can be a variety of solvents, blended together to extract the oil and, at the same time, react with the oil to produce the biodiesel. This is much more important when the catalyst present in liquid form. One needs to know how the droplet size distributions are related to the turbulent velocity when pressure variations, cause drops to break into smaller ones when the dynamic force exceeds the interfacial tension forces. The same drops may also collide with each other which may result in coalescence if the colliding droplets remain together for a long time enough to rupture the interfacial film between the two colliding droplets[47],

Ni and Pereira[48] investigated the droplet size distribution in a continuous oscillatory baffled reactor (COBR). It was acknowledged that the mixing in a COBR can be achieved by vortices generated when liquid oscillation is superimposed in a cylindrical tube containing periodically spaced annular baffles. The COBR used is shown in figure 2.9.2. During the experiment, three flowrates were used, corresponding to net flow Reynolds numbers of 250, 500 and 1000. As the fluid was oscillated, silicon oil was injected continuously.

The sampling was carried out at four different ports and was shown to be consistent between experiments. Microscope images were captured for droplet counting and drop size measurement. As this analysis was time consuming, the minimum number of drops was determined to provide representative and reliable size distribution for the given condition. These experiments were repeated to show repeatability of the results.

The analysis of this experiment indicated that, as the frequency of oscillation increased, the peak of droplet size distributions increased in height, indicating an increase in the fraction of the droplets at the lower end of droplet size range. The tail of the size distribution appears to get shorter, suggesting that more uniform droplets are formed giving a narrower distribution. This was explained by energy dissipation theory: as oscillation frequency increases, it increases the energy for dissipation, and hence the turbulence in the system, which favours the breakage rate over the coalescence, reducing average droplet size and narrowing the distribution. Increasing the amplitude has a similar effect as the oscillation frequency. The experimental results showed that the oscillatory motion plays a more dominant role in controlling the mean droplet

diameter than the net flow. It is the oscillatory motion superimposed on a baffled tube that produced and maintained 'plug flow like' distribution.

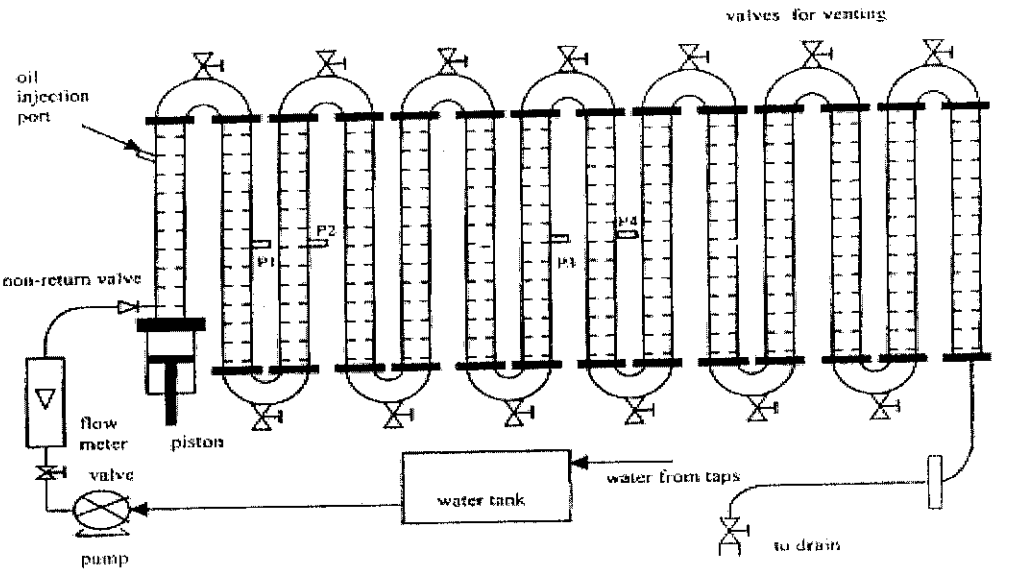


Figure 2.8.2: COBR Used for Droplet Size Distribution

2.9 LITERATURE REVIEW CONCLUSION

This review demonstrated the research in the area of sustainability and biodiesel production. Distributed economies concept is a potential way to create a sustainable biodiesel production since all the origin seeds are not transported out from the region but it is sent to a small scale plants built within the region. Therefore, it reduces pollution on transportation, increases local job and produces biodiesel at cheaper cost.

Carbon cycle for rapeseed oil as biodiesel fuels was investigated. This was to further prove the potential of biodiesel in reducing the global warming through the cycle of carbon. The biodiesel production was further reviewed on the kinetics of transesterification and the catalysts used in the process. The higher the molar ratio of alcohol to oil, the better the yield and the alkaline catalysts is better than the acid catalysts. Heterogeneous catalyst is a better solution in reducing waste in production. However, at this moment the used of this catalyst has a limitation on low yield and conversion.

The solvent used in vegetable oil extraction is further reviewed. Hexane is a common solvent used, but methanol and ethanol are other type of possible solvents where methanol has high selectivity and ethanol has high extraction capacity. This is important for in-situ transesterification because if there is a solvent that can also be used for reaction, the experiment is considered successful and can be further exploited.

Combining the oil extraction and reaction to produce biodiesel, known as in-situ transesterification was also studied. Previously, there were only two types of seeds that were used for in-situ transesterification related research: soybean and sunflower. The experiments were concluded successful and therefore can be further exploited.

This literature survey gave a good framework to begin the experiment. The experiment outlined will involve the combination of the extraction and reaction steps in batch processing. The variables here are the type of catalysts, their strength, and the solvent used for this process. The catalyst types include sodium hydroxide and sodium methodixe, and possibly some heterogeneous catalysts or supported enzymes.

Analysis will include the degree of conversion and percentage yield through thin layer chromatography analysis. Thus, operability and practicability of this process will be considered throughout.

If the process is considered practical, then the extraction and reaction of rapeseed oil in a continuous process will be carried out. Two types of reactors can be used: the oscillatory flow reactor (OFR) and a continuous stirred tank reactor (CSTR). Parameters of interests are the temperature, which ultimately determines the pressure and the agitation speed. The variation of agitation speed is to be implemented to study the interfacial area and suspension of seeds. Results from this stage may enable scale-up to pilot plant scale design.

3.0 EXPERIMENTAL METHODOLOGY

In this section, the experimental methodology, the materials and the equipment used are discussed. The four types of experiment that were carried out during the study are listed as below:

i) *In-situ transesterification / alcoholysis*

A combination of extraction and reaction of rapeseed in the present of hexane, alcohols and different catalysts with different strengths was carried out in batch process.

ii) *Liquid Membrane*

Emulsion liquid membrane (ELM) was created between hexane and methanol.

iii) *Re-extraction of Rapeseed*

The filtered rapeseed from in-situ transesterification was re-extracted using Soxhlet Extractor to prove Ryder's[49] theory.

iv) *Time Study*

Sampling was done during in-situ transesterification of rapeseed for specified time period. Further analysis was carried out to investigate the behaviour of the process.

3.1 MATERIALS

The materials used in the experiments within this study are tabulated as below:

Chemical	Grade	Source	Quantity
Hexane	98%	Sigma Aldrich	2.5 litre
Methanol	99.95%	-	
Ethanol	99.8%	-	
NaOH (pearls)	AR 99%	BDH	500 g
Rapeseeds	Oil Content: 41-44%	-	15kg
Span 80	S6760	Sigma Aldrich	250 ml
Sodium Methoxide Solution in Methanol	30% wt	Acros Organics	500 ml

Table 3.1.1: Materials Used for The Entire Experiments

3.2 EQUIPMENT

The equipment used in this experiment was divided in conjunction with the experiments stated previously.

3.2.1 In-situ Transesterification of Rapeseed

In this experiment, a grinder was used to grind the seeds into smaller flakes. Then, a three neck round bottom flask with a reflux condenser was used in the water bath for extraction and reaction of rapeseed. A shaking machine was used to provide mixing whilst a Buchner funnel was used in vacuum filtration unit to filter the solids meal out of the liquid solution. Finally, rotary evaporation unit was used to purify the extracts through solvent removal.

3.2.2 Liquid Membrane

In this experiment, a liquidiser was used to blend the solvent with the surfactant at the required amount to create an emulsion.

3.2.3 Re-extraction of Rapeseed Meal

As the seeds have been extracted, re-extraction of the seeds was carried out using the a Soxhlet extraction unit to extract the remaining oil in the meal. Then, the extracted oil was purified through solvent removal by using rotary evaporation unit.

3.2.4 Time Study

As for time study experiment, the same equipment was used as in experiment 3.2.1.

3.3 EXPERIMENTAL PROCEDURE

3.3.1 In-situ Transesterification of Rapeseed

Objective: Instead of extracting and purifying the oil from the seeds and reacting to produce biodiesel, the oil-bearing material was contacted directly with catalysed alcohol, indicating that alcohol now acts as both extraction solvent and transesterification reagent.

Materials: The chemicals used in this experiment were methanol and ethanol. Hexane was used as a co-solvent to assist in oil extraction. Sodium hydroxide (NaOH) acted as the catalyst and it was prepared in methanol solution according to its required strength. According to the stoichiometric reaction between methanol or ethanol and triglycerides, the amount of methanol and ethanol required for the transesterification reaction is 3 to 1. However, the amount of these alcohols shall be supplied in molar excess, i.e. more than 100% so that the forward reaction is favoured.

Methodology: 50 g of rapeseed was ground into smaller flakes using a grinder. Then, sodium hydroxide (NaOH) in methanol solution was prepared based on its required strength. According to the stoichiometric reaction, adding NaOH into methanol results in sodium methoxide (NaOMe) production:

$$NaOH_{(s)} + CH_3OH_{(l)} \rightarrow NaCH_3O_{(l)} + H_2O_{(aq)} \qquad \textbf{Equation 5}$$

The ground rapeseed was transferred into a round-bottomed flask together with hexane and methanol was added to achieve the ratio of methanol to hexane required. The reaction was carried out for one hour at 60°C in a water bath. As the extraction and reaction reached completion, the product was quenched in cold water and the remaining seeds were filtered out using vacuum filtration unit. Further purification was carried out to remove the solvent (hexane and unreacted methanol) using a rotary evaporation unit.

As for ethanol, the same procedures have been carried out with the exception that the catalyst was sodium ethoxide (NaC_2H_5O) via the following stoichiometric reaction:

$$NaOH_{(s)} + C_2H_5OH_{(l)} \rightarrow NaC_2H_5O_{(l)} + H_2O_{(aq)} \qquad \textbf{Equation 6}$$

For the experiment carried out using 30% wt of sodium methoxide in methanol, a simple calculation has been carried out to measure the exact amount of sodium methoxide required, and no further reaction occurred when diluting the sodium methoxide solution in excess of methanol.

The experimental setup for the whole experiment is as follows:

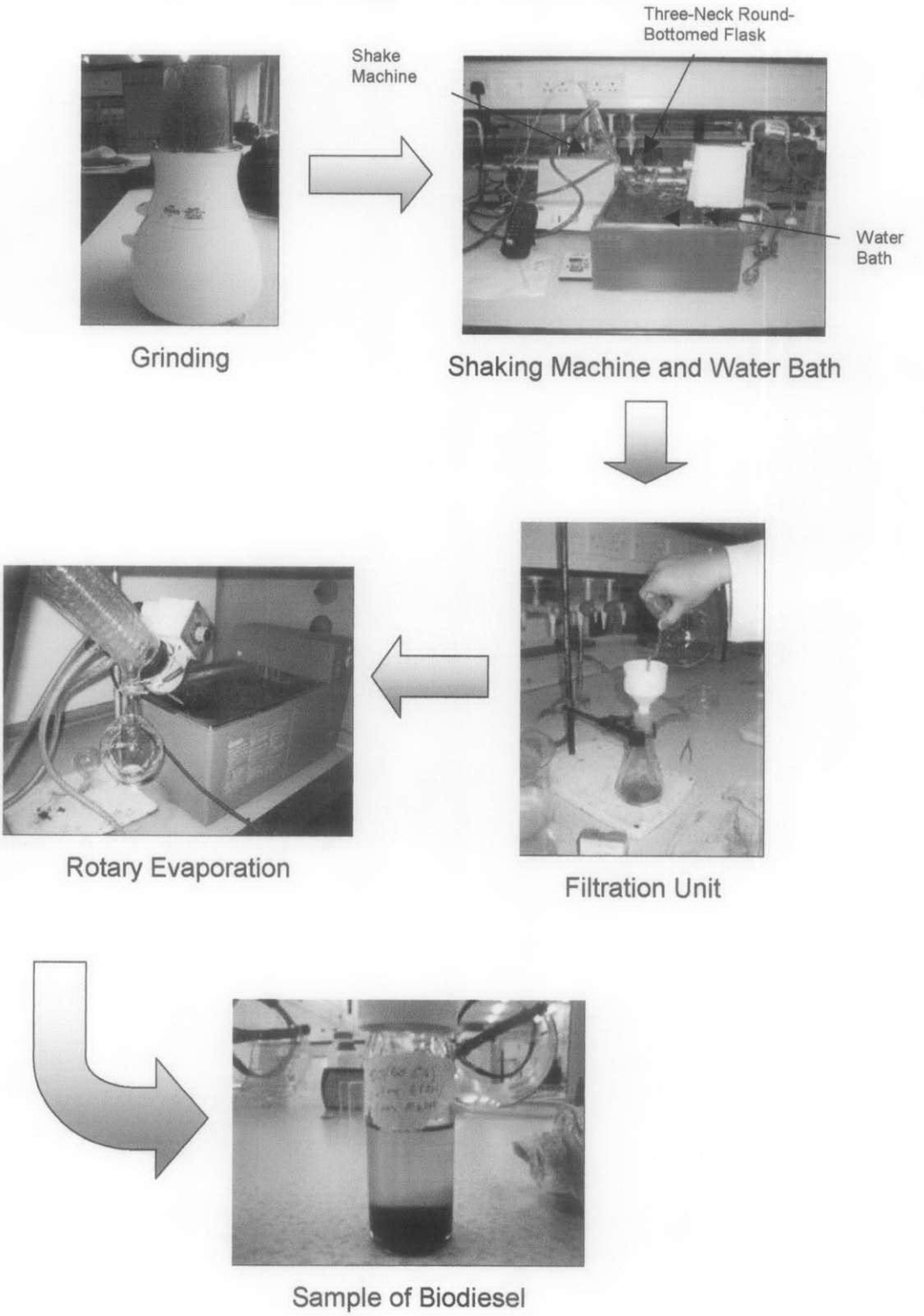


Figure 3.3.1: Experimental Set-up

3.3.2 Liquid Membrane

Objective: The literature indicated that in-situ transesterification required a large amount of alcohol for a complete reaction to take place. Therefore, this would be cost ineffective as typical transesterification reaction would require a molar ratio of 6:1 between the methanol and the triglyceride. Hence, creating an emulsion liquid membrane (ELM) using a discontinuous polar phase in continuous non-polar phase (hexane behaved as continuous non-polar phase and methanol behaved as discontinuous polar phase) ideally, would give a good result where a better solvent such as hexane could extract the oil and methanol contacted with the oil to produce biodiesel.

Material: Hexane was used in higher volume (vol %) ratio compared to NaOH solution in methanol. The surfactant used in this experiment was Span 80 (Sigma Aldrich) since to create water in oil emulsions; the surfactant should have hydrophilic-lipophilic balance (HLB) value in the range of 3-6.

Methodology: Two alternative sequences those were carried out to produce the emulsion liquid membrane.

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1st Route:

Hexane of approximately 100 ml was poured into the liquidiser altogether with a tablespoon of Span 80. Then, approximately 10ml of NaOH solution in methanol of strength 1.0 M (1.3m) was added slowly whilst mixing took place and the mixing was stopped after 10 minutes.

2nd Route:

Instead of adding the hexane, 10 ml of NaOH solution in methanol of strength 1.0 M (1.3m) was poured into the liquidiser first. Then, the surfactant was added and blended at the same amount as before, and about 100 ml of hexane was added slowly whilst the mixing took place. The reaction was stopped within the same duration.

Whether this is an emulsion can be determined by its appearance. Typical emulsions are turbid.

3.3.3 Re-Extraction of the Leftover Seeds

Objective: M Ryder[50] in his report assumed that some of the methanol solution has been adsorbed by the seeds during in situ transesterification. This is the reason why at low amount of methanol, even at optimum ratio of methanol to triglyceride (6:1), no biodiesel has been produced. This experiment therefore has been conducted to justify the assumption.

Material: Two types of seeds were used for the re-extraction. One was left to dry overnight and re-extracted (dry meal) and another one was re-extracted directly after filtering the seeds and the products (wet meal). 400 ml of hexane was used for every re-extraction.

Methodology:

Dry seeds re-extraction

After leaving to dry overnight, the dry seeds have been reweighed and a portion of it was filled into a thimble and weighed. The thimble was placed into a Soxhlet container where approximately 400 ml of hexane had been put into the round-bottomed flask and connected to the Soxhlet container. A flask heater was used to heat up the hexane until it vaporised (b.p of hexane is 69°C). A reflux condenser was used to ensure the vaporised hexane would return back into the Soxhlet container in liquid form. The hexane therefore will extract the oil from the seeds and the extracted oil altogether with the solvent will fall down to the round bottom flask due to the siphon effect as hexane reached the Soxhlet container maximum liquid height. This phenomenon will repeat by itself. The extraction was considered completed if the colour of the liquid in the Soxhlet container was the same as hexane i.e. a clear liquid. The Soxhlet apparatus is shown in Figure 3.3.2.

Wet seeds re-extraction

The procedure was the same as for dry seeds re-extraction except that instead of drying the seeds overnight, the seeds was directly re-extracted once it had been filtered out from the products.

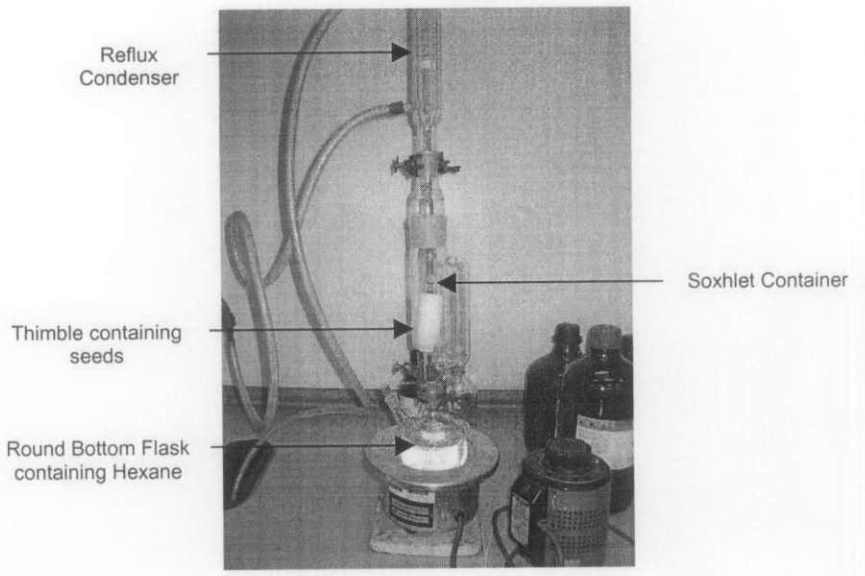


Figure 3.3.2: Soxhlet Apparatus

3.3.4 Time Study Experiment

Objective: A time study was carried out to investigate the extraction and reaction behaviour along the process. From literature, it was expected that in-situ alcoholysis would increase the amount of oil dissolved, by increasing the reaction time.

Material: NaOH in methanol solution was prepared for strength of 0.1 molality and it was contacted with 50 g of rapeseed. No hexane was used in this experiment. Glacial acetic acid (17M) was used to quench the reaction.

Methodology: 50 g of rapeseed was ground and put in the round-bottomed flask. This flask was located in the water bath prior to addition of the solution. 0.1 m NaOH in methanol was added and the clock started once the mixing began. The extraction and reaction occurred for 1 hour but for every period, eleven sampling had been carried out by using pipette and the sample was directly quenched by using approximately 0.1ml of acetic acid glacial (17M) as to stop the triglyceride reacting further. The samples were directly analysed using thin layer chromatography (TLC) method.

3.4 ANALYTICAL METHOD

The analytical methods were carried out to analyse qualitatively and quantitatively the samples obtained from all the experiments carried out within the duration specified. Two main analyses were used: material balance and thin layer chromatography (TLC).

3.4.1 Material Balance

Objective: The material balance was carried out thoroughly for the entire runs. Therefore, better approximation of biodiesel yield could be obtained.

Methodology: The block diagram of the whole process is shown in Figure 3.4.1

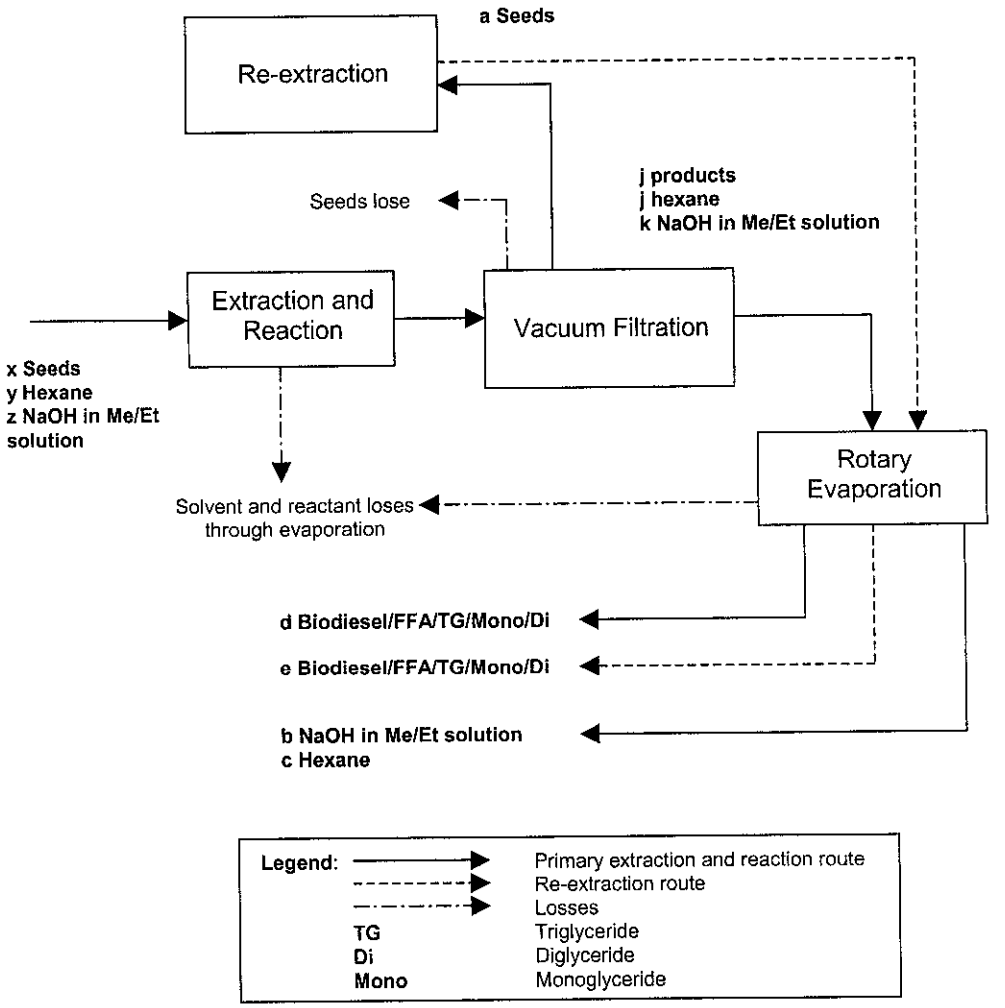


Figure 3.4.1: Block Diagram of The Process

When the reaction was stopped after one hour extraction and reaction, filtration took place where the left out seeds, the product and the losses incorporated with the seeds were weighed. Then, the product was purified by using rotary evaporator. The solvents recovered were weighed as was the final yield. The seeds were then dried overnight in the fume cupboard and reweighed the next day.

In the re-extraction process, only a portion of the seeds was used and reweighed before the extraction. After re-extracting the seeds, the seeds were reweighed and left to dry overnight, and reweighed again. The solvent and product recovered from the re-extraction experiment were reweighed and purified through rotary evaporation. Then, the extract and solvent recovered were reweighed.

3.4.2 Thin Layer Chromatography (TLC)

Objective: Fast and reliable results could be obtained from the analysis made by thin layer chromatography (TLC). Therefore, this method is a quick way to observe and quantify the conversion of biodiesel produced through in-situ transesterification of rapeseed.

Material: The material used are listed below

Material	**Grade**	**Source**	Quantity
Petroleum Ether	Laboratory Reagent	Fisher Scientific	**2.5 l**
Diethyl Ether	99.5%	BDH	**2.5 l**
Acetic Acid Glacial	17 M, AR 99.7%	Fisons	**2.5 l**
TLC Plates (20 cm)	0.25mm Silica Gel	Sigma Aldrich	**50 plates**
Iodine	Crystals	-	**10**
Pure Biodiesel Methyl Oleate	99%	Sigma Aldrich	**2.5 l**
Rac-1-Oleoyl-Glycerol	~40% (TLC)	Fluka	**1 kg**
Filter paper	**20 cm diameter**		

Table 3.4.1: Material Used for Thin Layer Chromatography Analysis

Methodology: Initially, a mixture of petroleum ether, diethyl ether and acetic acid glacial with volumetric ratio of 85:13:1.5 (vol %) was prepared for plate development. Then, two filter papers were cut and located inside the TLC chamber containing 20 ml of the

solvent mixture as above. When the filter papers were fully soaked, the silica plate was placed inside the TLC chamber to allow the plate being soaked as well. 1 ml of the sample from the experiment was withdrawn and mixed up with 5 ml of petroleum ether in another sampling bottle. Once the plate was pre-soaked (the solvent mixture travelled at about 12 cm onto the surface of the plate from the bottom), it was dried and the sample which has been primed earlier was applied onto the plate by using a glass capillary tube of 10 μl. After the sample on the plate dried, the plate was located back into the chamber and the overall development took about 15 minutes. The plate was soaked up again until it reached 12cm, and put to dry outside the chamber for another 2-3 minutes. In order to visualise the pattern developed on the plate, the plate was transferred into another chamber containing 10 iodine crystals. A clear image can be obtained if the plate was allowed prolong in the chamber with iodine.

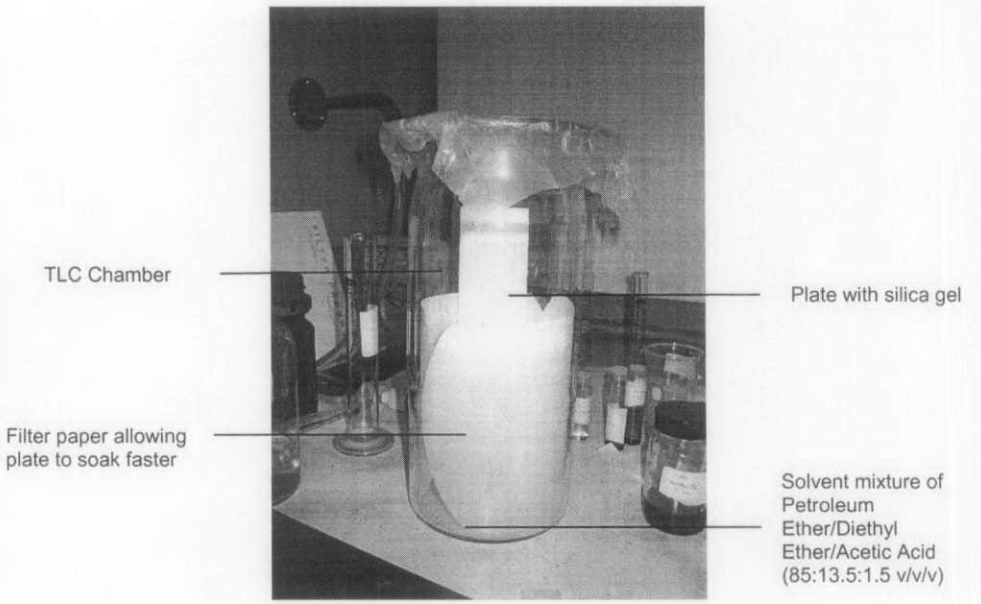


Figure 3.4.2: TLC Equipment and Material

Criterion of detection: Once the plate was developed, identification of the blobs appeared on the plate could be done for further quantitative analysis. Based on literature review and benchmark analysis, each component in the sample was represented by the retention time i.e. each component travelled on the plate at different distance. This is shown in the next page. The identification could also be done as follows:

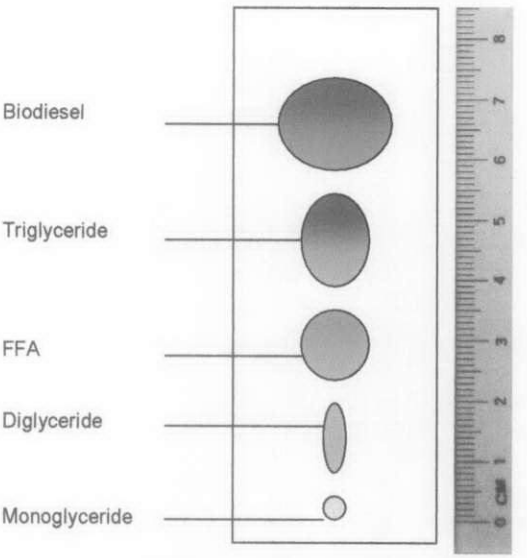


Figure 3.4.3: Detection of Product's Components on TLC Plates

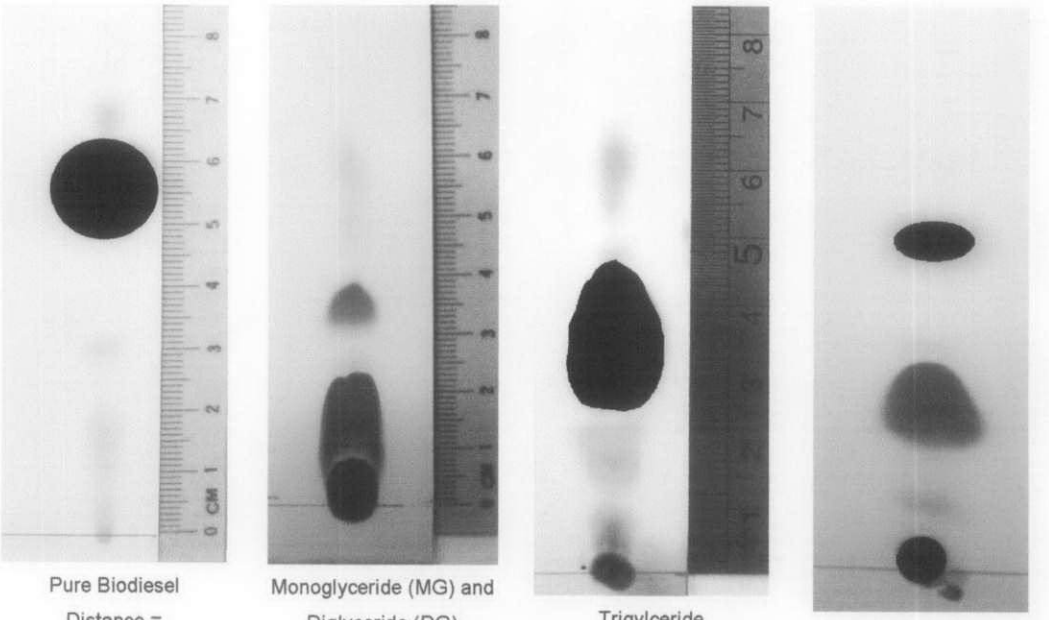


Figure 3.4.4: Retention Time of Different Components on TLC Plates

Quantitative Analysis from TLC: The image developed on the plate was captured by a digital camera. A ruler was located beside the plate to assist in measurement. By using *Image J* software, developed by NIH Image, a fixed scale was set at 114 pixel/cm.

Then, the area of biodiesel, triglyceride (TG), monoglyceride (MG) and diglyceride (DG) were measured respectively. The response factor in this analysis is the area fraction, where the area of biodiesel's blob is divided by total area of biodiesel, MG, DG and TG. By referring to the calibration curve, which shall be discussed next, the conversion of biodiesel area can be determined.

Calibration Curve Construction: Ryder[51] in his report had constructed a calibration curve based on triglyceride and biodiesel as his response factor. This curve is reliable on giving the biodiesel weight percentage (wt %) at lower percentage (i.e. less than 50 vol%) of the ratio of methanol to hexane since at these particular ratios, the amount of triglyceride was considerably high at any catalyst strength. However, if the ratio of methanol to hexane (vol%) is higher than approximately 50%, the curve was not accurate since the area response factor did not correspond to the area of monoglyceride (MG) and diglyceride (DG). This resulted in biodiesel wt% to achieve 100% whilst in actual, it was not.

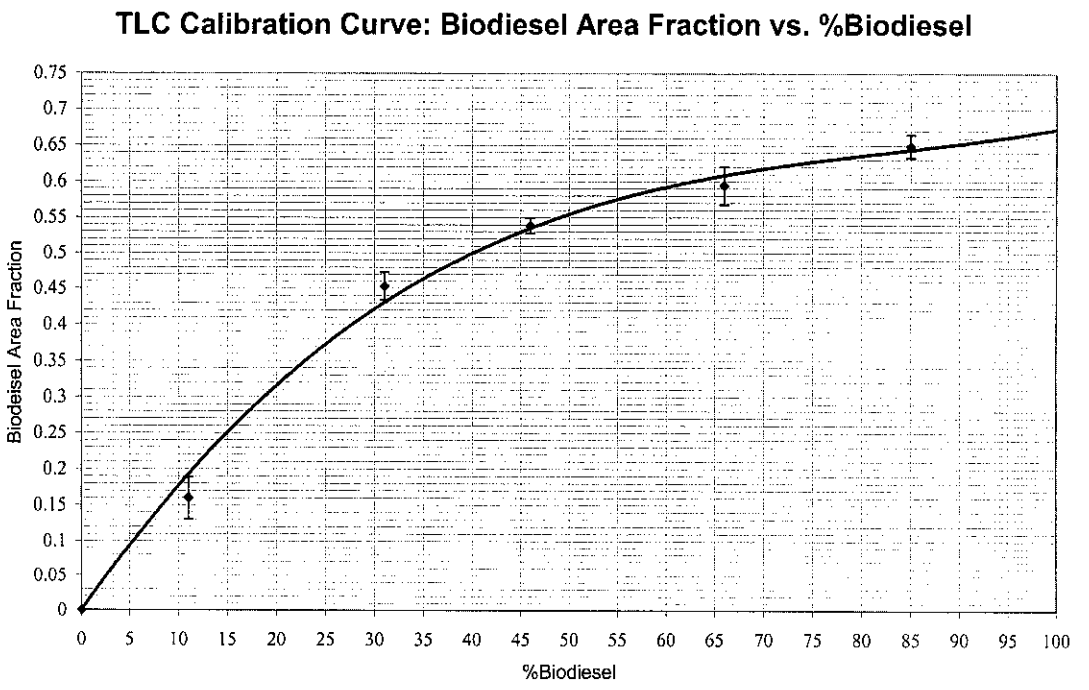


Figure 3.4.5: Ryder's Calibration Curve on Biodiesel Area Fraction vs % Biodiesel

A solution known as Rac-1-Oleoyl-Glycerol, contained approximately 40% of monoglyceride, about 40% of diglyceride and 20% of triglyceride was used for another

calibration curve construction. Initially, two plates were used to determine whether these glycerides' composition corresponded to their area evolved on the plate. The test concluded that these glycerides matched up to their respective area as response factors.

Further calibration was carried out by mixing the solution with pure biodiesel (methyl oleate) at respective ratio. These samples was developed onto the plates and also aimed for reproducibility. The biodiesel area was therefore divided by total area of biodiesel, monoglyceride and diglyceride to obtain its area fraction and the data points were plotted on the graph. An example of calculating the area fraction of biodiesel from Image J measurement is as follow, taking 60 wt% data point:

Component	**Area Plate 1**	**Area Plate 2**	**Average Area**
Biodiesel	1.644	1.856	1.75
Diglyceride	0.467	0.541	0.504
Monoglyceride	0.258	0.369	0.3135
Total Area	2.369	2.766	2.5675
Area Fraction of Biodiesel	0.6940	0.6710	0.6825

Table 3.4.2: Calculations of Area Fraction of Biodiesel

The final graph for five sets of data points is shown as Figure 3.4.6:

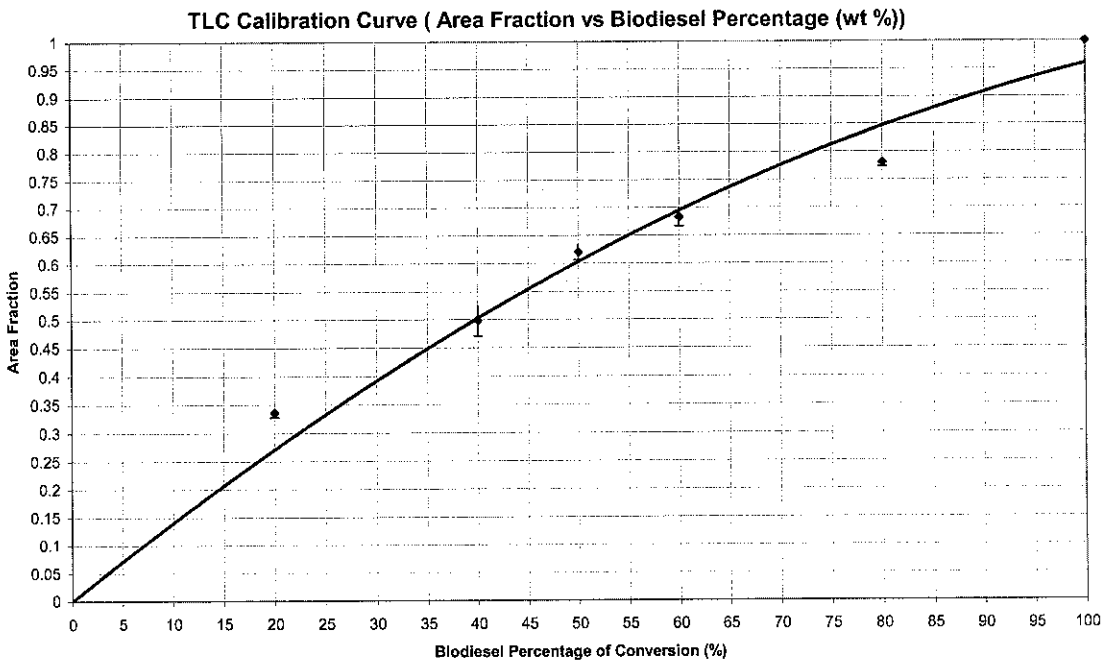


Figure 3.4.6: Modified Calibration Curve of Biodiesel Area Fraction vs Biodiesel Yield Percentage (wt%)

TLC Error: Random error was included in this curve and it was calculated by taking standard deviation of two fractional biodiesel areas. As an example, 60 wt% biodiesel sample random error calculation is shown below:

Area Fraction (FA): 0.6940, 0.6710

Average Area Fraction (A) : 0.6825

Random Error:

$$\varepsilon_r = \sqrt{\frac{1}{2}\sum (FA_i - A)^2} = \sqrt{\frac{(0.694-0.6825)^2 + (0.671-0.6825)^2}{2}} = 0.016$$

In average, the random error in measurement was in between 0.8% to 3%

4.0 RESULTS AND ANALYSIS

In this section, the results from the experiments carried out for the entire study was shown here. Discussions on each experimental result are made to explain the phenomenon or occurrences along the entire experiments.

4.1 IN-SITU TRANSESTERIFICATION OF RAPESEED

There were 38 runs in different conditions which were tested and further analysed during the entire experiment. Each run took about 1 and half hour until the sample can be collected. This included grinding, extraction and reaction, vacuum filtration and purification by solvent removal. All runs were tabulated as below:

Type of Alcohol	Catalyst Type	Catalyst Strength/ molality (m)	Alcohol to Hexane Ratio	Volume of Solvent/ Amount of Seed (ml/g)
Methanol	Sodium Hydroxide (NaOH)	0.5	60/40	250/50
			80/20	250/50
			100/0	250/50
		0.1	10/90	200/30
			20/80	200/30
			30/70	200/30
			40/60	200/30
			50/50	200/30
			60/40	200/30
			70/30	200/30
			80/20	200/30
			100/0	200/30
			100/0	250/50
		0.05	10/90	250/50
			20/80	250/50
			30/70	250/50

			40/60	250/50
			50/50	250/50
			60/40	250/50
			80/20	250/50
			100/0	250/50
Ethanol		0.5	10/90	250/50
			20/80	250/50
			30/70	250/50
			40/60	250/50
			50/50	250/50
			60/40	250/50
			100/0	250/50
		0.1	40/60	250/50
			50/50	250/50
			100/0	250/50
		0.05	10/90	250/50
			50/50	250/50
			100/0	250/50
Ethanol and Methanol		0.1	50/50 (Ethanol/Methanol)	250/50
Methanol	Sodium Methoxide (NaOMe)	0.5	100/0	250/50
		0.1	100/0	250/50
		0.05	100/0	250/50

Table 4.1.1: Runs Designed for In-situ Transesterification of Rapeseeds

For reaction involved 0.5m NaOH in methanol, the runs were limited since it was a continuation from Ryder's work. More runs were carried out using methanol compared to ethanol due to its availability and a projection of further work concentrated on methanol only. Sodium methoxide was used to replace sodium hydroxide so that a comparison could be made between these two catalysts. In 0.1m NaOH in methanol, only 200 ml of total methanol and hexane was used for 30g of seeds so that the effect

of ratio of methanol to seeds in term of conversion and yield of biodiesel could be studied.

4.1.1 Methanol and NaOH

The degree of strengths of NaOH in methanol was tested in the experiment. Solution of NaOH in methanol was prepared based on molality (m) i.e. number of moles of NaOH divided by 1 kg of methanol to ensure it is consistent with Ryder's[52]. The results obtained from three degrees of strengths of NaOH in methanol are plotted as below. This included Ryder's results on 0.5 m NaOH in methanol.

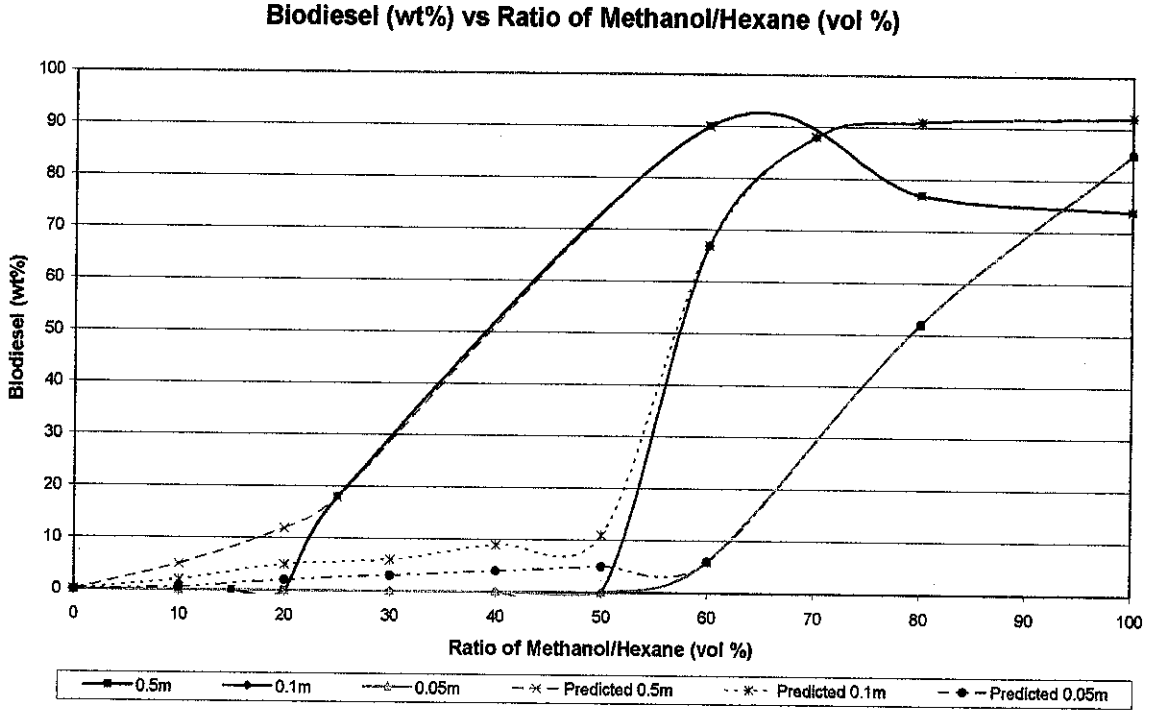


Figure 4.1.1: Biodiesel (wt%) vs Ratio of Methanol/Hexane (vol%)

An overall plot yielded some interesting results to be discussed. As expected, at lower ratio of methanol to hexane, almost no biodiesel was produced. As the ratio of methanol to hexane increased, biodiesel was produced with respect to the catalyst strength.

For 0.5 m NaOH in methanol, the highest yield percentage was achieved at methanol to hexane ratio of 65:35, which is approximately about 92%. This is the same as produced by 100% 0.1m NaOH in methanol. At 0.5 m strength, higher methanol to hexane ratio

resulted in the yield to drop until it reached approximately 78%. Moreover, at ratio of methanol to hexane started from 60/40 up to 100/0, there was very small amount of liquid phase produced, and other than that it was totally a brownish solid, wax look. Further analysis using TLC indicated that high content of free fatty acid was produced. This phenomenon was believed to come from the amount of catalyst used, which was way too high and therefore converted as water and further saponified leading to soap formation. Naik[53] demonstrated that the optimum amount of alkaline catalyst for biodiesel production was 1 wt% based on triglycerides amount i.e. the oil. Taking condition of 60/40 (methanol/hexane) ratio of 0.5 m NaOH in methanol, which used 150 ml of the solution, the exact amount of catalyst used was 2.36 g. Shahidi[54] in his publication highlighted that oil content in typical rapeseed was between 41.7-43.2% and 8.5% out of it was moisture content. From 50 g of rapeseed, it was assumed only 21 g of triglyceride could be extracted and 1.79 g of it was moisture content. This leaves only 19.21 g of triglyceride. Comparing 2.36 g catalyst compared with 19.21g of oil resulted in 12.28 wt% of catalyst end up being used.

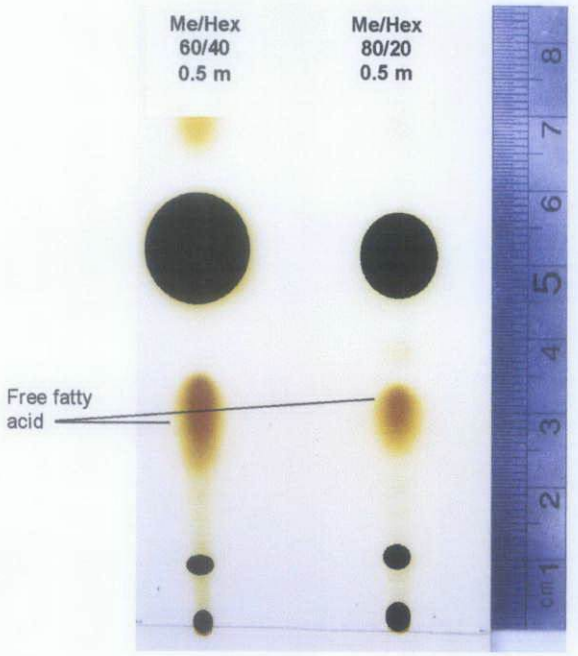


Figure 4.1.2: The Presence of Free Fatty Acid at 0.5 m NaOH in Methanol

Using 0.1 m of NaOH in methanol was assumed in the region of appropriate catalyst strength since it used only 4.1 wt% of the catalyst. The product was less viscous at

higher methanol to hexane ratio indicated that most of the triglycerides were converted as biodiesel and intermediary products (monoglycerides and diglycerides). The higher the amount of methanol, the higher yield percentage of biodiesel obtained. This suggested that the molar ratio of methanol to triglycerides played an important role in optimising the production.

It is believed that at lower amount of methanol, there should be some biodiesel produced. However, TLC method cannot detect any of it. The predicted curve based on catalyst strength was depicted in the plot too. This will be further discussed in re-extraction results and analysis.

At lower strength of catalyst (0.05 m of NaOH in methanol), the amount of catalyst used was assumed the best strength. Even though the percentage yield of biodiesel was low than 0.1 m NaOH in methanol, by using the same amount of rapeseeds (50 g), the conversion in 100% 0.05 m NaOH in methanol reached 83% whilst 100% 0.1 m of NaOH in methanol only reached 63%.

A study was also conducted on the ratio of alcohol to seed in biodiesel production. Four runs were carried out: two runs with 30 g of seeds and 200 ml of methanol (0.1 m NaOH) and another two were 50 g of seeds and 250 ml of methanol. The ratios were 6.67 and 5 respectively. It was found that the yield percentage of biodiesel did not change much in each run (90-92%) but the conversion from triglyceride to biodiesel was high when using 30g seeds and 200 ml methanol. The extracted yield from 30g of seeds reached 92% conversion from theoretical extract, and for 50g seeds, the conversion was only 63%.

4.1.2 Ethanol and NaOH

Apart from investigating the methanol, ethanol was used and the catalytic solution was prepared with the same strength as methanol solution. This experiment was carried out so that some comparisons could be made between methanol and ethanol performance. However, since time and material were limited, not all ratios of ethanol to hexane could be tested.

The results are plotted in Figure 4.1.3:

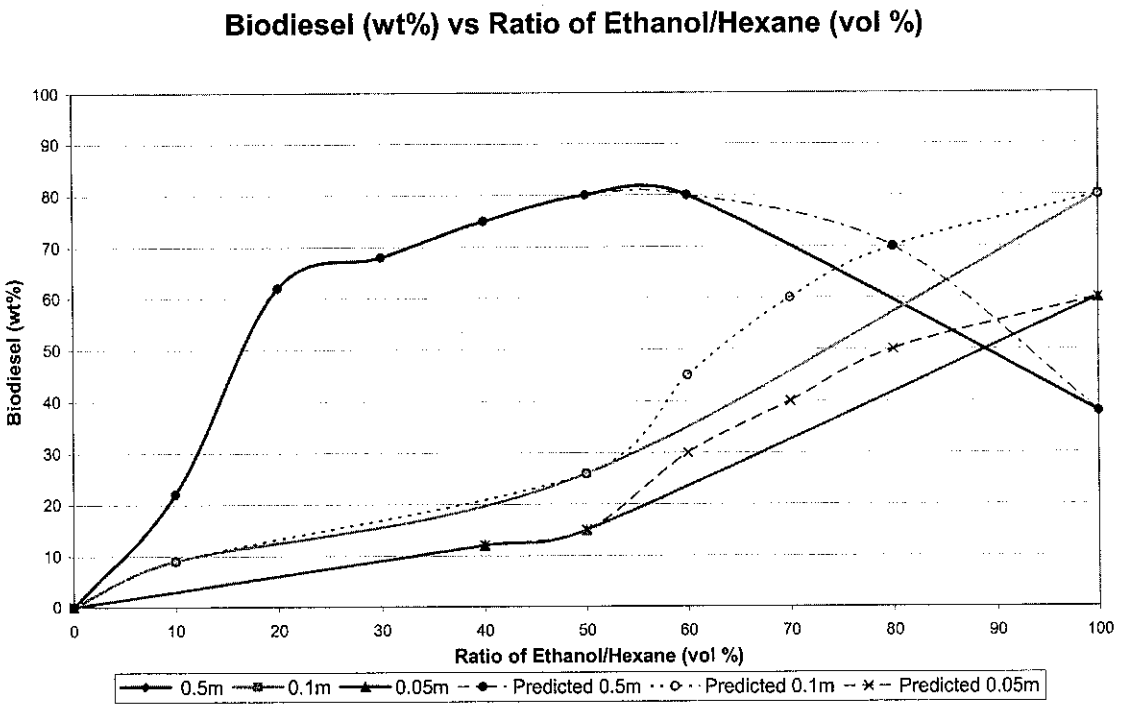


Figure 4.1.3: Biodiesel (wt%) versus Ratio of Ethanol/Hexane (vol%)

Looking at overall picture of these data points, almost the same pattern of results were obtained in ethanol study. However, most of the NaOH solutions in ethanol yielded biodiesel at lower ratio of ethanol to hexane but at higher amount of ethanol, lower yield percentage of biodiesel were achieved compared to using methanol alone.

From the observation, NaOH of 0.5 m strength in ethanol produced almost the similar result as NaOH of 0.5 m in methanol. For lower ratio of ethanol to hexane, biodiesel had already been produced at high yield percentage. However, some brownish and greenish solid phase mixture was formed at ratio of 30/70 up to 100% ethanol. This was believed due to excess amount of catalyst and therefore other products have formed instead of biodiesel alone, which resulted in greater amount of free fatty acid (FFA). Even though there was about 80 wt% biodiesel produced in 50/50 solution; this was useless as solid phase dominated the final product form. The purification of the product through rotary evaporation became tougher as the separation was very volatile. A better proof of FFA existence can be made based on the TLC plates as shown in Figure 4.1.4:

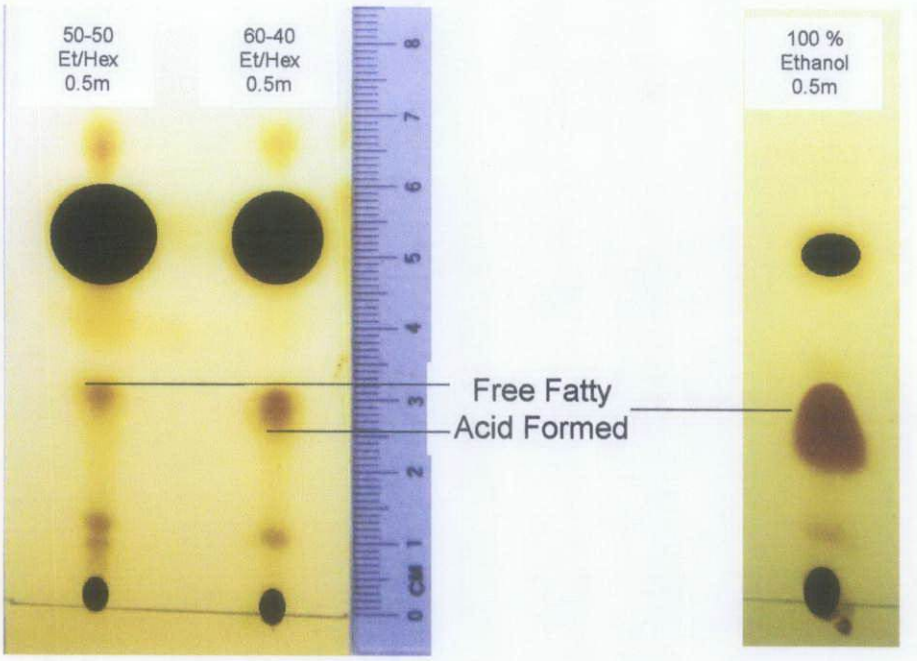


Figure 4.1.4: Free Fatty Acid Formed on The Plates of 0.5 m NaOH in Ethanol

For 0.1m and 0.05m NaOH in ethanol solution, biodiesel was already produced at lower ratio of ethanol to hexane, compared with 0.1m and 0.05 m NaOH in methanol. However, as the ratio increased, the yield percentage was lower than the methanol solution. This suggested that the ethanol is a better solvent than methanol where small amount of ethanol could assist the extraction of triglycerides by hexane and straight away reacted with the oil extracted to produce biodiesel. This was different with methanol since at low hexane to methanol ratio, reflecting a low molar ratio of methanol to triglycerides; almost no biodiesel was produced based on TLC plate's observation. The case was different at higher methanol to hexane ratio, where a higher yield percentage of biodiesel was achieved in comparison to ethanol, suggesting that methanol is a better reactant. TLC plates for both low and high hexane to alcohol ratio were depicted as in Figure 4.1.5:

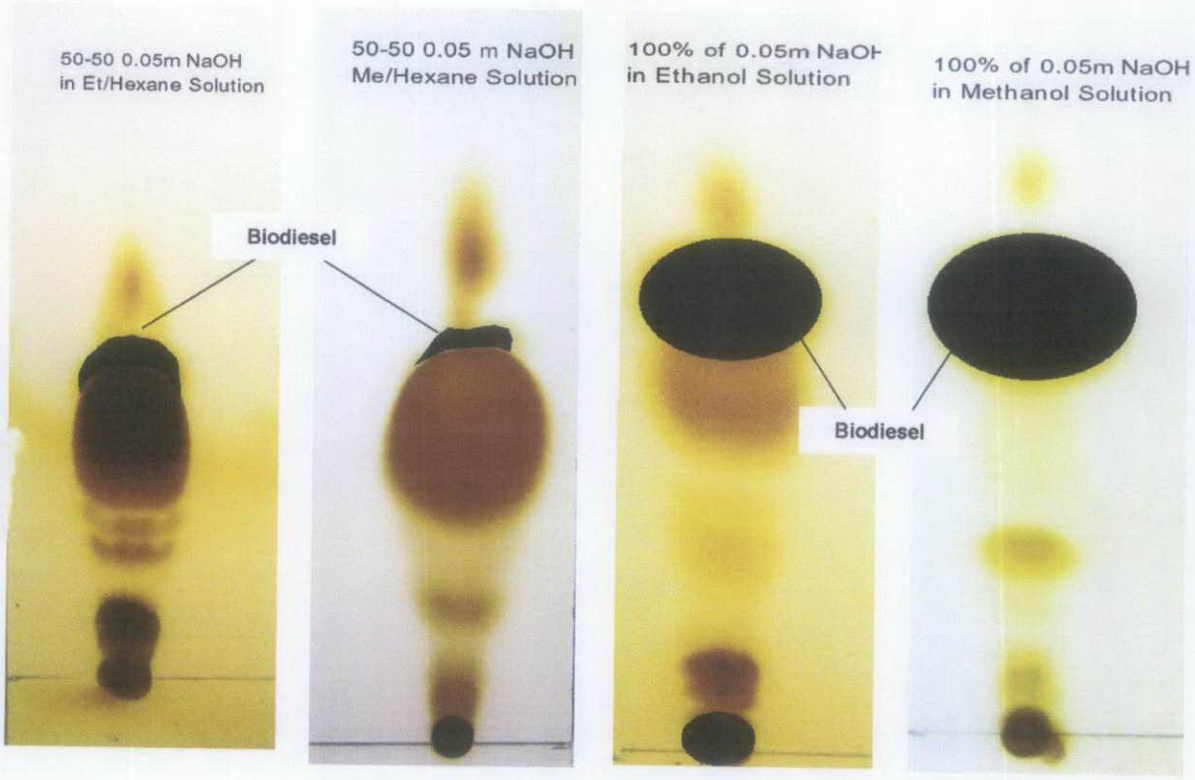


Figure 4.1.5: High and Low Ratio of Alcohol to Hexane for Ethanol and Methanol

At low ratio of alcohol to hexane, it was obvious that more biodiesel was produced by ethanol but based on the alcohols alone i.e. 100% 0.05 m NaOH in alcohols, greater yield percentage of biodiesel obtained from methanol solution. This confirmed the literature demonstrated by Meirelles[55] on equilibrium data for ethanol and methanol in canola oil. Ethanol is a good solvent since the extraction capacity is bigger than methanol whereas methanol is a good reactant since it has high selectivity, thus choosing only the right component to be reacted, hence letting methyl ester production as its primary reaction.

4.1.3 Sodium Methoxide and Sodium Hydroxide

From the experiments, a comparison was made on three samples of NaOH in methanol solution, and three samples of NaOMe in methanol solution. The results are as follows:

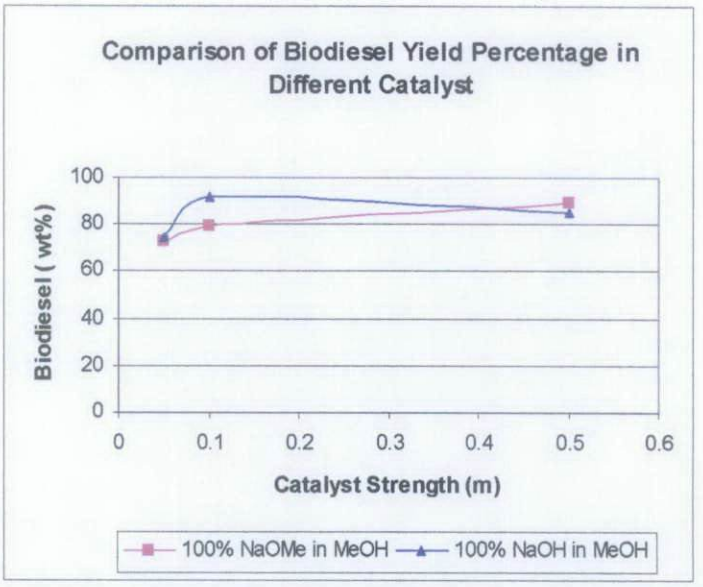


Figure 4.1.6: Comparison of Biodiesel Yield Percentage In Different Catalyst

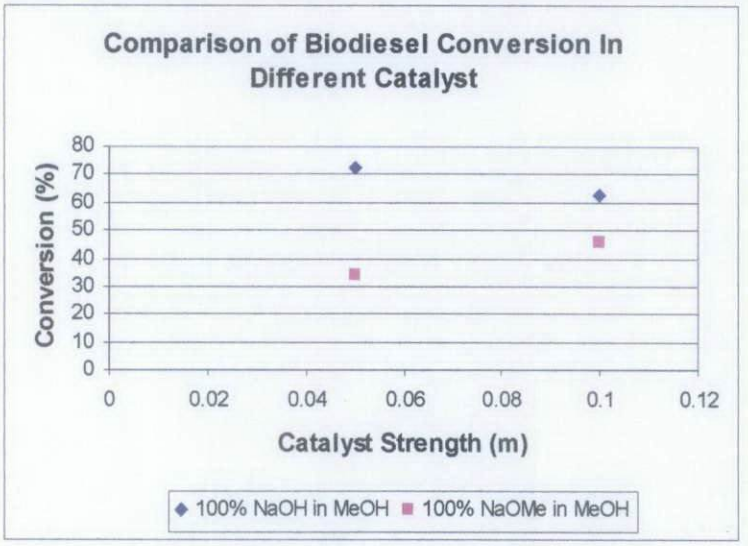


Figure 4.1.7: Comparison of Biodiesel Conversion in Different Catalyst

From the graph, it was obvious that by using NaOH, the biodiesel yield percentage and conversion was higher compared to sodium methoxide. By increasing the concentration of the NaOH from 0.05m to 0.1m, which was approximately 2.05 wt% to 4.09 wt%, the yield of NaOH reached almost 92% for 1 hour extraction and reaction, whilst for NaOMe, the yield only increased from 73% to 79%. For the same catalyst strength, the percentage of NaOMe in wt% with respect to the triglycerides was higher than NaOH. For 0.05 m NaOMe, the catalyst used was 7.9 wt% whilst for 0.1 m, the concentration was 15.8 wt%. Eventually, as the concentration was further increased 5 times stronger,

the yield percentage of biodiesel using NaOMe was higher than biodiesel using NaOH. It is believed that when NaOH was used, the extraction and reaction occurred faster than NaOMe and therefore, higher percentage of yield was obtained. For NaOMe, the extraction and reaction was slower, hence there were some other intermediates in the product when it lasted for 1 hour reaction (refer to Figure 4.1.8)

In terms of the biodiesel conversion, two samples of NaOH in methanol resulted in high conversion of biodiesel whilst NaOMe has lower biodiesel conversion. This conversion was calculated by dividing the yield of biodiesel by theoretical triglyceride amount, which was about 42% from the total rapeseeds used. Further analysis showed that for NaOH in methanol solution, the conversion of biodiesel decreased as the strength of catalyst increased. However, this was the opposite case for NaOMe in methanol solution. For both catalyst strength of 0.5 m, the conversion could not be determined because it was believed that there was a competing reaction occurring at the same time of transesterification, which resulted in extraction yields were higher than the theoretical amount.

These results concurred with Aracil *et al*[56]. Aracil showed that, if using NaOH, biodiesel was converted the fastest in comparison with KOH, KOMe and NaOMe. Sodium has high dissociation constant and apart from that methoxide anion produced from the reaction of NaOH and methanol is dependent on the hydroxide ion amount, which is the catalyst concentration i.e. NaOH. As sodium molecular weight is low, more hydroxide ion thus is required to produce methoxide ion. In different with NaOH, NaOMe did not dependent at all on hydroxide ion and the amount of methoxide ion generated from sodium methoxide was lower than the amount of methoxide produced by sodium hydroxide. Therefore the reactants using NaOMe converged later. This explained why NaOH was the fastest convergence catalyst. However, there were more impurities produced when NaOH was used, resulted from NaOH reaction with methanol, which produced water and NaOMe, Therefore, saponification reaction occurred and produced high content of free fatty acid (FFA). This is the reason why as catalyst strength of NaOH increased, more impurities presented and therefore saponification took place rather than transesterification. A plate comparison was made based on these two types of catalysts.

From the plates, it was obviously that by using NaOMe, less FFA was produced and this is indicated by the total area of the blobs representing it. This result was in line with the one concluded by Aracil *et al*[57] and it was proven by simple calculations as follows:
From stoichiometric equation **(Equation 1)** on NaOH and methanol,

$$NaOH_{(s)} + CH_3OH_{(l)} \rightarrow NaCH_3O_{(l)} + H_2O_{(aq)}$$

The ratio of water to sodium methoxide is 1:1. For 250 ml solution of 100% 0.05 m NaOH in MeOH, 0.393 g of NaOH is required, which is equivalent to 0.01 moles. This would produce approximately 0.6 g of NaOMe and 0.18 g of water. Apart from that the seed itself contained 1.785 g of moisture in case if 50 g of rapeseed is used. Comparing this with pure NaOMe, the water obtained from the rapeseed was only half the amount obtained by using NaOH. This is the reason why there was high amount of FFA in the solution which used NaOH as catalyst.

It is suggested that if pure NaOMe is to be used for this reaction and extraction, the reaction time should be allowed longer and it is expected that the yield of biodiesel will be the same as if NaOH catalyst is used. The catalyst strength should not however, exceed 0.1 m.

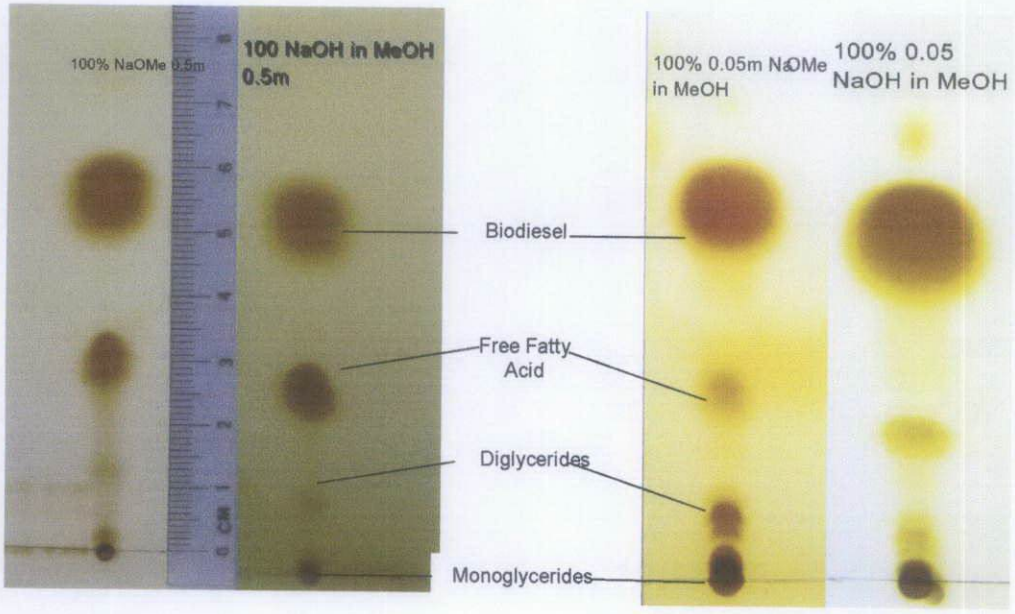


Figure 4.1.8: Comparison of NaOH and NaOMe at High and Low Catalyst's Strengths

4.1.4 Biodiesel Conversion

From this experiment, the conversion of biodiesel was also investigated. The results are represented as Figure 4.1.9:

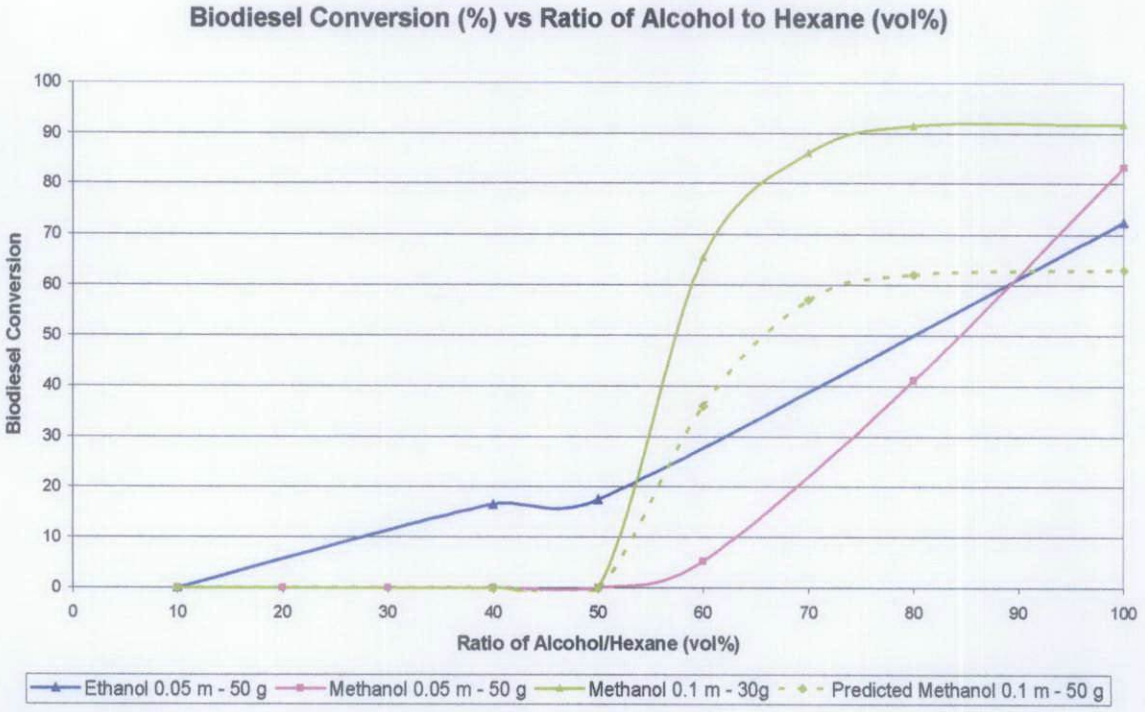


Figure 4.1.9: Biodiesel Conversion (%) vs Ratio of Alcohol to Hexane (vol%)

The above results depicted the biodiesel conversion versus the ratio of alcohol to hexane. The biodiesel's conversion percentage was calculated by multiplying the extract with the percentage of biodiesel (wt%) and divided by theoretical amount of triglyceride expected from the seeds, and finally multiplied by 100%. The amount of rapeseed used was either 30 g or 50 g. The plot indicated that the biodiesel conversion behaved differently for different type of alcohols, the catalyst strengths and ratio of alcohol to seeds used. Note that in the graph discussed earlier on different type of catalysts, for results of 100% 0.1 m NaOH in methanol, the amount of rapeseeds used was 50 g and this was not the same as the above plot (30g). However, for comparisons purpose, a predicted curve for 0.1 m NaOH in methanol of 50 g seeds was plotted too. At lower ratio of alcohol to hexane, biodiesel conversion for ethanol was higher than methanol. When biodiesel was formed at higher ratio of methanol to hexane, the conversion however was greater than ethanol.

Looking at the details, ethanol gave lower conversion at higher ratio of ethanol to hexane (vol%) compared to methanol. This was probably resulted from the behaviour of the alcohols as discussed earlier where ethanol is a better solvent and methanol is a better reactant. At lower ratio of alcohol to hexane, there was no biodiesel formed in methanol solution but there was some when ethanol was used. It was assumed that the ethanol assisted hexane in extracting the oil and straight away reacted to produce biodiesel. Since ethanol has low selectivity i.e. it extracted everything out from the seed and therefore allowing other reactions to occur, there was an excess concentration on the product side and thereby reduced the extraction capability of ethanol. Hence, at higher ratio of ethanol to hexane, there was a reduction in extraction and therefore low yield of biodiesel. However, this was different compared to methanol in both 0.1 m and 0.05 m concentration. Since methanol is a better reactant than ethanol, the conversion was quite high at high methanol to hexane ratio. This resulted from extracted triglyceride, which reacted straight away with methanol to produce biodiesel. High selectivity behaviour of methanol reduced the amount of triglyceride extracted since methanol reacted with it and thus more triglyceride was extracted according to Le Chatelier's Principle. Nonetheless, at low ratio of methanol to hexane, it could not support hexane in extraction due to its limitation of low extraction capacity.

In comparing the ratio of methanol to the seeds, the higher the amount of alcohol with respect to the seeds amount, the higher the conversion it would be. This was in correspondence with Ryder's[58] explanation on single extraction stage experiment which stated that increasing the amount of the solvent will increase more extract. However, not all runs and samples can give correct amount of yield since some of the losses occurred during rotary evaporation.

4.1.5 NaOH in Combination of Ethanol and Methanol Solution

From previous results, ethanol and methanol were combined together so that ethanol could extract the triglycerides whilst methanol could react to produce biodiesel. 0.1 m of NaOH was prepared in both ethanol and methanol solution since from the previous experiments indicated that the optimum results could be obtained from 0.1 m strength. The result obtained from this experiment was very good where the biodiesel extract was 12.47 g, which was about 65% conversion. This was higher than 100% 0.1 m NaOH

solution in methanol of 50 g seeds, where the conversion was only 63%. Two distinctive layers existing on the product, which was assumed as biodiesel and also glycerol.

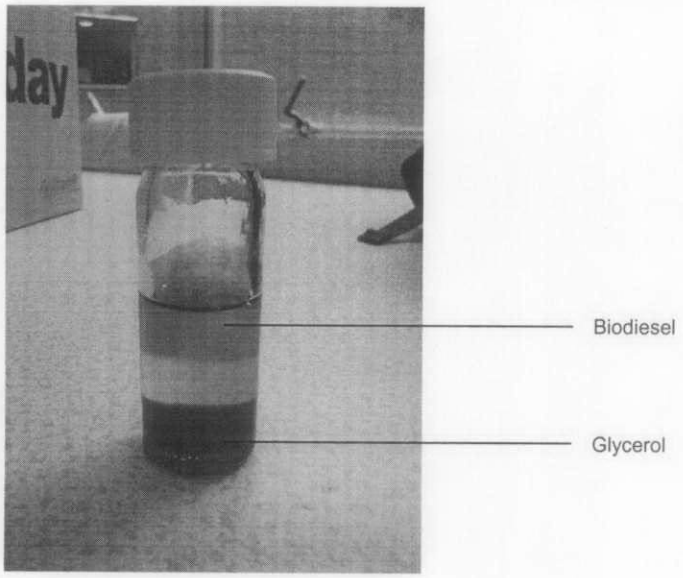


Figure 4.1.10: 50/50 0.1 m NaOH in Methanol and Ethanol Solution

4.2 LIQUID MEMBRANE

In this experiment, three runs were tested as follows:

Run	Route	Hexane to Methanol Ratio (v/v)
1	1	75:25
2	1	50:50
3	2	75:25

Table 4.2.1: Runs on Liquid Membrane Sequencing

Based on the observation, no emulsion was successfully created for these three runs. Two distinctive layers persisted whilst Span 80 dissolved in methanol even though the surfactant should be able to create water in oil type of emulsion. An example of this behaviour is shown in Figure 4.2.1.

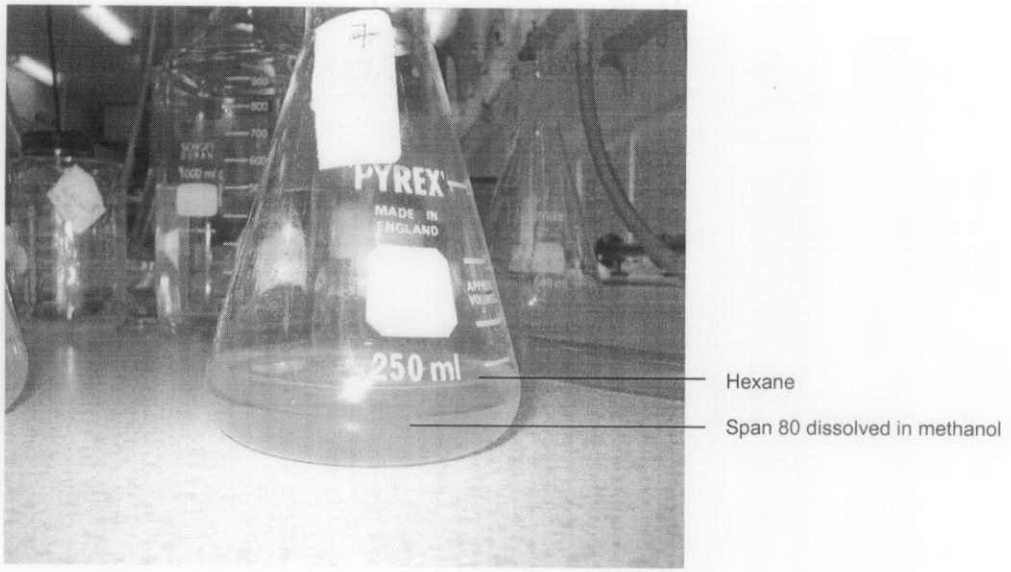


Figure 4.2.1: Sample of Liquid Membrane Experiment (Run 2)

Tao and Chen[59] demonstrated that in order to create stable emulsion, the HLB value of the material to be emulsified; in this case methanol should have the same HLB value with the material to emulsify i.e. the surfactant. However, the information of methanol's HLB value was unobtainable at this moment.

Based on experiment on 4.1.1 and 4.1.2 on methanol, ethanol and NaOH catalysts, it can be concluded that instead of having methanol at low amount to be emulsified, it is worth while to emulsify ethanol instead of methanol. This resulted from the behaviour of methanol and ethanol at low ratio of alcohol to hexane, in which methanol did not produce any biodiesel at low amount of methanol but ethanol managed to produce some.

4.3 RE-EXTRACTION OF RAPESEEDS MEAL

Four runs were carried out as part of re-extraction of leftover rapeseeds. These runs, which used pure hexane for re-extraction purposes are shown as follows:

Run	Ratio Of Methanol To Hexane During Extraction/Reaction	Catalyst	Type of Re-extraction
1	100/0	NaOH 0.1 m	Dry
2	100/0	NaOH 0.1 m	Wet
3	10/90	NaOMe 0.1 m	Wet
4	30/70	NaOMe 0.05 m	Wet

Table 4.3.1: Runs on Re-Extraction of Leftover Rapeseeds

Out of these four runs, the extract recovered from run 3 and run 4 were very little after re-extraction took place. Furthermore, there was only one layer existed on solvent recovery flask after rotary evaporation. Plate analysis could not be carried out since the amount recovered was very small. It was believed that most of the triglycerides had been extracted during in-situ stage and therefore no more triglyceride could be obtained during re-extraction.

For run 1 and run 2, it was observed that no detectable distinctive layers appeared on these runs during rotary evaporation took place i.e. after re-extraction stage. Significant amount was recovered during re-extraction for both runs. Direct re-extraction (wet seeds) process extracted approximately 11.33% out of the leftover seeds whilst indirect re-extraction (dry seeds) extracted 6.55% out of it. This was highly possible since on wet seeds, there might be some product leftover on the surface of the seeds and adsorbed by the seeds whilst for dry seeds, it was well understood that the seeds was very dry and no other components on the seeds' surfaces. Thus, in wet re-extraction, it was most likely that the extracts were obtained from the surface and inner side of the seeds and for indirect re-extraction, the extracted components came from only the inner side of the seeds.

Another important fact was that both re-extraction processes yielded biodiesel out of it, which have almost the same yield percentage (~83%). This is shown as follows:

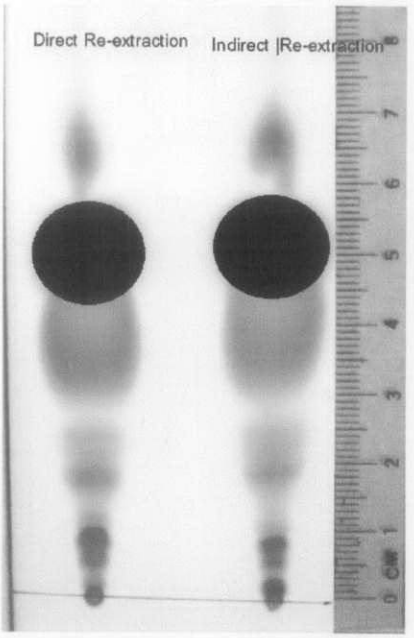


Figure 4.3.1: Wet (Direct) and Dry (Indirect) Re-extraction

The above phenomenon disproved Ryder's[60] theory, which stated that methanol was adsorbed by the seeds since there was no distinctive layer presented during separation stage. This was further proven by the fact that both direct and indirect re-extraction gave out similar biodiesel's yield percentage, noting that methanol is highly volatile and it can vaporise once left to dry overnight. However, it could be stated that actually some of the biodiesel was adsorbed by the seeds, hence during re-extraction, no further reaction occurred but the biodiesel adsorbed was released into the solvent flask.

4.4 TIME STUDY

There were 2 runs carried out during this experiment. Both were from the same solution, i.e. 0.1 m NaOH solution in methanol for reproducibility. This run was chosen since from previous experiment indicated that 0.1 m NaOH in methanol gave good conversion and higher yield percentage.

Twelve samples were taken out along 1 hour experiment: 3 minutes, 6 minutes, 9 minutes, 10 minutes, 12 minutes, 15 minutes, 20 minutes, 25 minutes, 30 minutes, 40 minutes, 50 minutes and 60 minutes. The results are shown as follows:

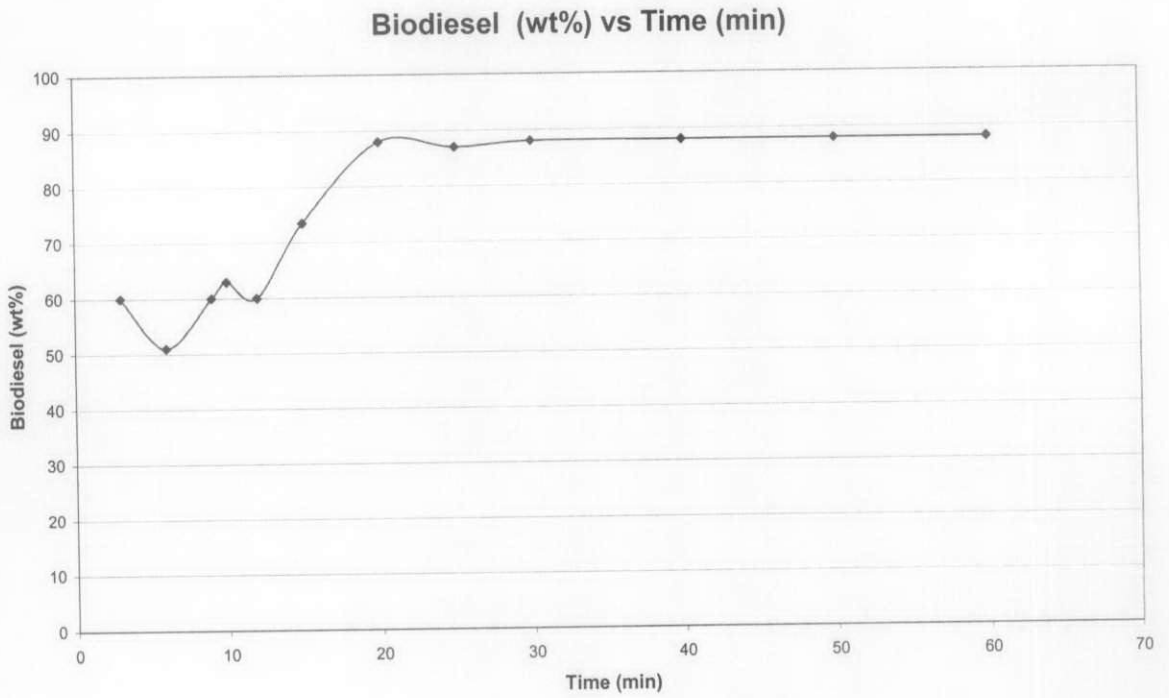


Figure 4.4.1: Biodiesel Yield Percentage (wt%) vs Time (min)

From the above plot, it can be shown that in the initial period, the yield percentage of biodiesel was not quite high and unstable. By the time it reached 20 minutes during extraction and reaction, the biodiesel yield percentage became constant at 88% to 89% and this can be considered as steady state condition. It was assumed that initially, methanol acted as solvent to extract the triglycerides out from the seeds. Once extracted, it reacted straight away and form biodiesel and intermediates

(monoglycerides and diglycerides) at low yield and concentration. According to Le Chatelier's principle, a low concentration in product side will allow the extraction became faster and more triglycerides were extracted and reacted with biodiesel. These occurred simultaneously and therefore for the first 15 minutes, the process was not really stable where an excess or shortage of triglycerides occurred and hence there were fluctuations of biodiesel's yield. The process slowly became stable and as extraction occurred, the reaction also occurred at uniform rate, hence producing more biodiesel within the reaction time at specific yield percentage. This behaviour can be showed as follows:

$$TG(seeds) \rightarrow [TG(oil) \Leftrightarrow Biodiesel]$$

Extraction Occurred Continuously — Reaction Occurred Continuously at Equilibrium Rate

More biodiesel was produced at constant composition (wt%) along the time and this could be further proof by observing the colour of the biodiesel's spot on the plates. As time increased, the spot representing biodiesel became darker as shown in the diagram depicted in Figure 4.4.2:

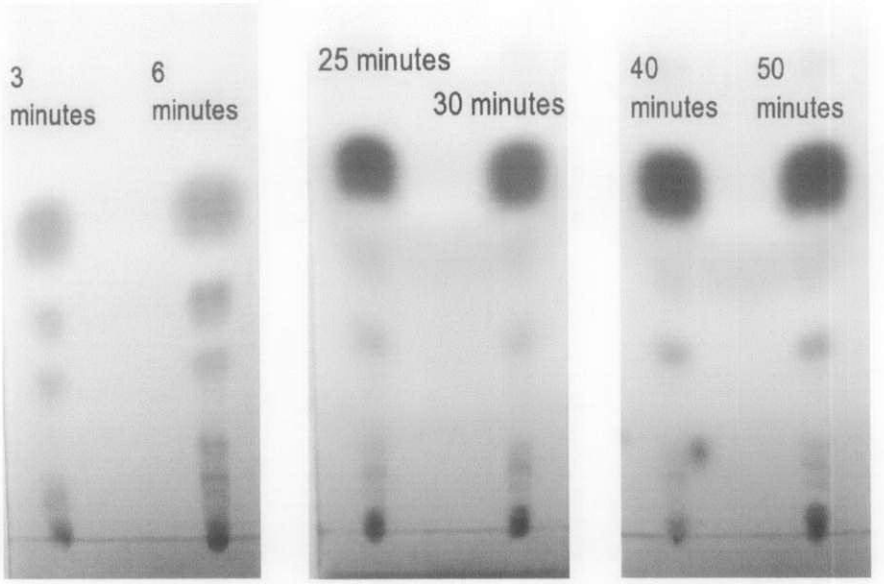


Figure 4.4.2: Plate's Pattern with Changing Time

Further experiment carried out by Guanrong[61] can be used to show that the extracted yield increased as time increased, which supported previous results. This can be shown as follows:

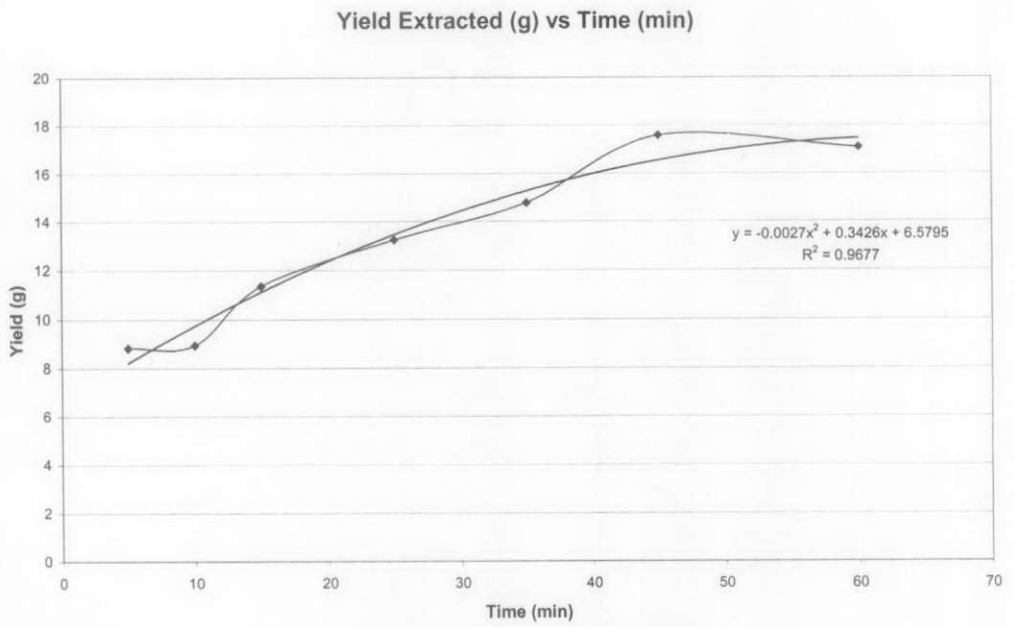


Figure 4.4.3: Yield Extracted (g) vs Time (min)

A trend line was plotted to predict the amount of extract that could be obtained theoretically for time study experiment. It can be concluded that even though the yield percentage becomes constant, the extracted yield will increase as time for extraction and reaction becomes prolong. The predicted conversion of biodiesel for this process is shown as follows:

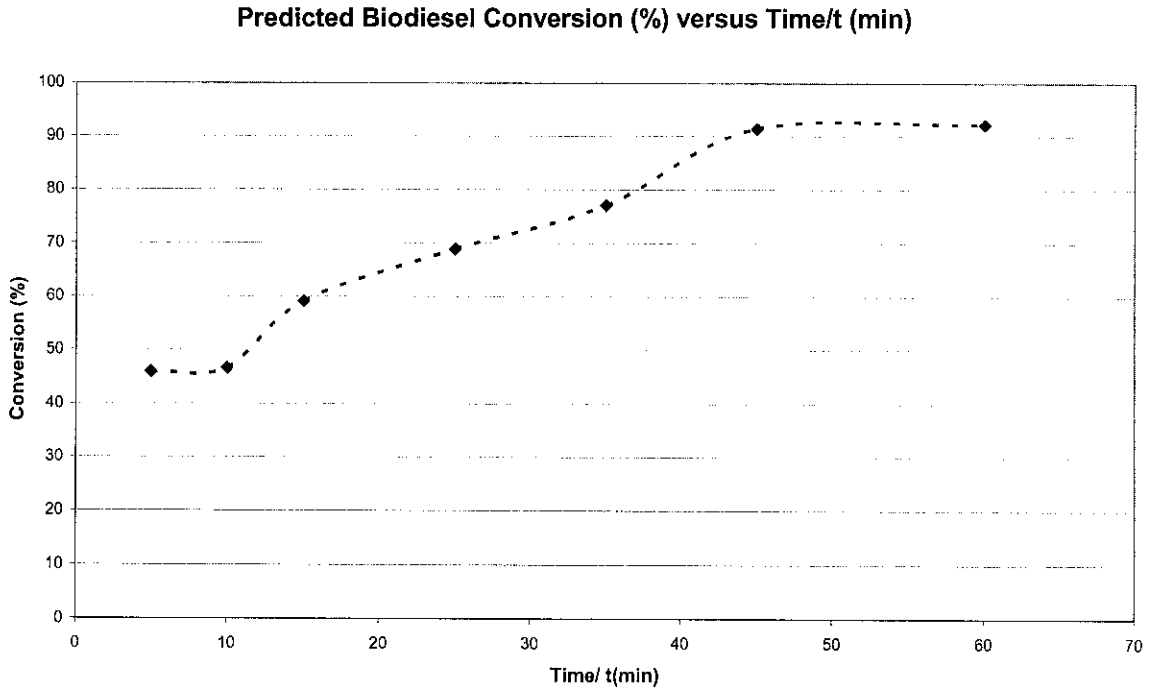


Figure 4.4.4: Predicted Biodiesel Conversion (%) versus Time (min)

The biodiesel conversion was predicted to increase as time increased (Figure 4.4.4). The triglyceride reacted straight away once extracted and the conversion increased rapidly. However, once it achieved higher conversion, the gradient decreased gradually.

There are three assumptions why biodiesel was not converted up to 100%.

1. Short reaction time, in this experiment only up to 1 hour reaction. Longer reaction time is suspected to give better degree of conversion.
2. A competing reaction occurred for example saponification, which leads to soap formation, hence producing impurities.
3. Error related to TLC analysis.

The kinetics of the reaction can be correlated once time study experiment is completed. However, a better analytical method such as gas chromatography is required to determine the exact composition in each sample at particular time, thus giving the correct concentration of biodiesel and other side products, hence more accurate kinetic of reaction can be determined.

5.0 CONCLUSIONS

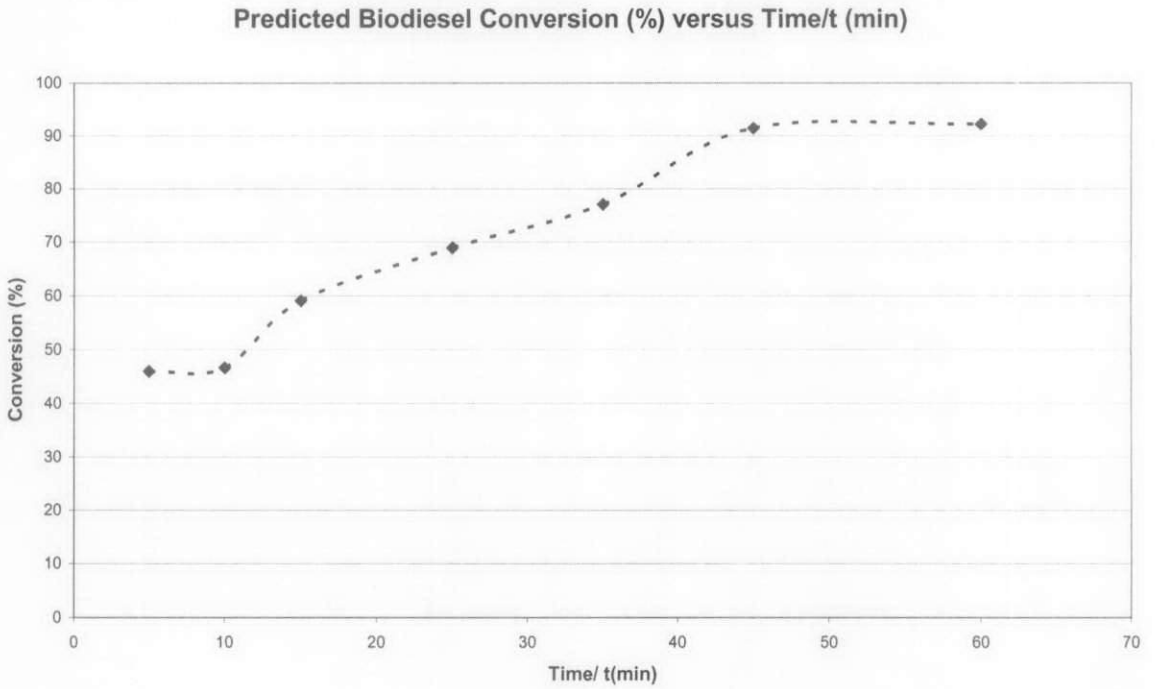
There are a lot of conclusions for the experiments could be carried out during this study. In-situ transesterification of rapeseed is possible and therefore has a high probability of commercialisation. However, the optimum conditions of catalyst strength, type of catalyst, type of alcohol, molar ratio of alcohol to seeds and molar ratio of alcohol to hexane (if to be used), need to be determined before deciding the right condition on larger scale pilot plant reaction.

Catalyst strength plays an important role in getting the right product and amount of extract which produces high yield of biodiesel. A small amount of alkaline catalyst would result in a small amount of biodiesel being produced within the specified reaction time whereas a large amount of alkaline catalyst would result in saponification leading to soap formation. This is because the catalyst used, in this experiment was NaOH can produce high amount of water once reacted with methanol and water present is very critical in the reaction. 0.1 m for both methanol and ethanol, using NaOH was already sufficient to produce high yield and high yield of biodiesel.

The type of catalyst especially the alkaline catalysts, used in this experiment was also a critical factor in getting the best results. Using NaOH would allow biodiesel to be produced at high yield faster than NaOMe. However, high amount of NaOH would affect the whole product as more FFA will be produced from the reaction.

It was also inferred that methanol is a better reactant and ethanol is a better solvent. Some biodiesel produced at low ratio of ethanol to hexane and higher yield percentage of biodiesel was obtained at higher ratio of methanol to hexane. Thus, these two alcohols were expected to produce high yield percentage of biodiesel and high degree of conversion. Hence, methyl ester and ethyl ester production will become two competing reactions in this process, and possibly reducing other impurities.

The amount of alcohol used for this process was also critical since a small amount of alcohol; though it satisfied the stoichiometric reaction; did not produce any biodiesel.



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The kinetics of the reaction can be correlated once time study experiment is completed. However, a better analytical method such as gas chromatography is required to determine the exact composition in each sample at particular time, thus giving the correct concentration of biodiesel and other side products, hence more accurate kinetic of reaction can be determined.

up to the expectation. It was thought that biodiesel conversion was higher than what have been shown before. Random errors of 0.8% to 3% persisted when measuring the spots on the plates. Apart from that, developing the plates and identifying the spots on the plates caused some other errors. Consistency of pattern emerged on the plates and reproducibility of the same spots can also be doubted.

Overall it is clear that in-situ transesterification is possible and could be commercialised. However, further work has to be carried out on small scale test to make sure that the production is fully optimised.

6.0 FURTHER WORK

Further works on this study are recommended as follows:

6.1 Investigate the property and behaviour of methanol and ethanol if to be combined together by varying the ratio of methanol to ethanol at concentration of not greater than 0.1 m of NaOH solution. This is an extended experiment for what have been carried out before by using 50/50 (v/v %) solution of 0.1 NaOH in methanol and ethanol.

6.2 Investigate the usage of heterogeneous catalysts therefore reducing the separation cost and avoiding waste from resulting saponification reaction.

6.3 Analyse the samples using gas chromatography method instead of using thin layer chromatography to obtain precise composition in the product quantitatively.

6.4 Investigate the ability to combine this project with gasification since bioethanol which is produced by gasification can be further used to produce biodiesel. Akay *et al*[62] stated that biodiesel represents a partial short term solution to a sustainable energy technology since the biodiesel feedstock is essentially a food grade material. However, the picture is more promising for bioethanol production from lingocellulose is considered.

6.5 Examine the ability of ethanol to be emulsified if hexane is still required to be used as main solvent for extraction. The surfactant to be used hence needs to have almost the same HLB value with ethanol. However, elimination of one solvent i.e. hexane will reduce cost of raw material used in this process and therefore separation of the product.

6.6 Analyse the samples thoroughly using various tests especially to detect phospholipids which is harmful to the fuel engine.

6.7 Analyse the meal obtained from the filtration to confirm this meal has high amount of protein and also less or free of glucosinolates; a sulphur-containing compounds which hydrolyse to produce toxic substances, and the bitterest phenolic constituents of the seeds. Diosady[63] suggested that washing with methanol, water and ammonia could

eliminate glucosinolates. Therefore, this would produce high quality meal, which gives additional revenue on top of biodiesel production.

6.8 Obtain an optimum condition including type of alcohol, type of catalysts, optimum temperature and residence time for the best results and sustainability consideration. This shall include cost analysis, environmental and social benefit.

6.9 Investigate how oscillatory flow reactor (OFR) can be used as intensified equipment in this process. Running on pilot plant scale means a need to investigate the operating conditions such as temperature, pressure and also agitation speed.

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8.0 APPENDIX

8.1 CALCULATION

8.1.1 Conversion of Biodiesel

$$Conversion = \frac{\frac{Yield\_Percentage\_Of\_Biodiesel\ (wt\%)}{100} \times Extract}{Theoretical\_Yield\_Expected}$$

8.1.2 Preparation of Sodium Methoxide in Methanol (250 ml)

Density of NaOMe, $\rho = 0.96\,{}^{g}/_{cm^3}$

Mass for 1 L of NaOMe, $m = \rho v = 0.96 \times 1000 = 960g$

Molecular Weight of NaOMe, $M_w = 54.02$

Given that NaOMe was 30wt% in methanol, $0.3 \times 960g = 288g$

Number of moles of NaOMe, $n = \frac{288}{54.02} = 5.33\_moles$

Molality of NaOMe in 1 kg methanol,m $\frac{5.33}{(960-288)g} = \frac{x}{1000}$

$x = 7.93m$

Therefore, for 0.1 m in 1L solution $0.1m = \frac{1000ml}{79.3} = 12.61ml$

For 100% 0.1m NaOMe in methanol (250ml) $\frac{12.61}{4} = 3.153ml$

8.1.3 Preparation of Sodium Hydroxide (NaOH) in Methanol (250 ml)

Density of Methanol, $\rho = 0.792\,{}^{g}/_{cm^3}$

Molecular weight of NaOH, $M_w = 40.00$

1 L of methanol has mass of $m = \rho v = 0.792 \times 1000 = 792g$

40 g of NaOH in 1 L methanol is equivalent to $= \frac{1}{0.792} = 1.26m$

Therefore, for 0.1 m NaOH in 1 L methanol, the NaOH required $x = \frac{0.1 \times 40}{1.26} = 3.174g$

For 100% 0.1m NaOH in methanol solution (250 mL), NaOH required was $= \frac{3.175}{4} = 0.794g$

8.1.4 Percentage of Catalyst in wt% With Respect to the Oil Amount

Amount of Oil in Rapeseed (Theoretical) = 42 wt% from total rapeseeds
Moisture Content in Rapeseed Oil (Theoretical) = 8.5 wt% from total oil

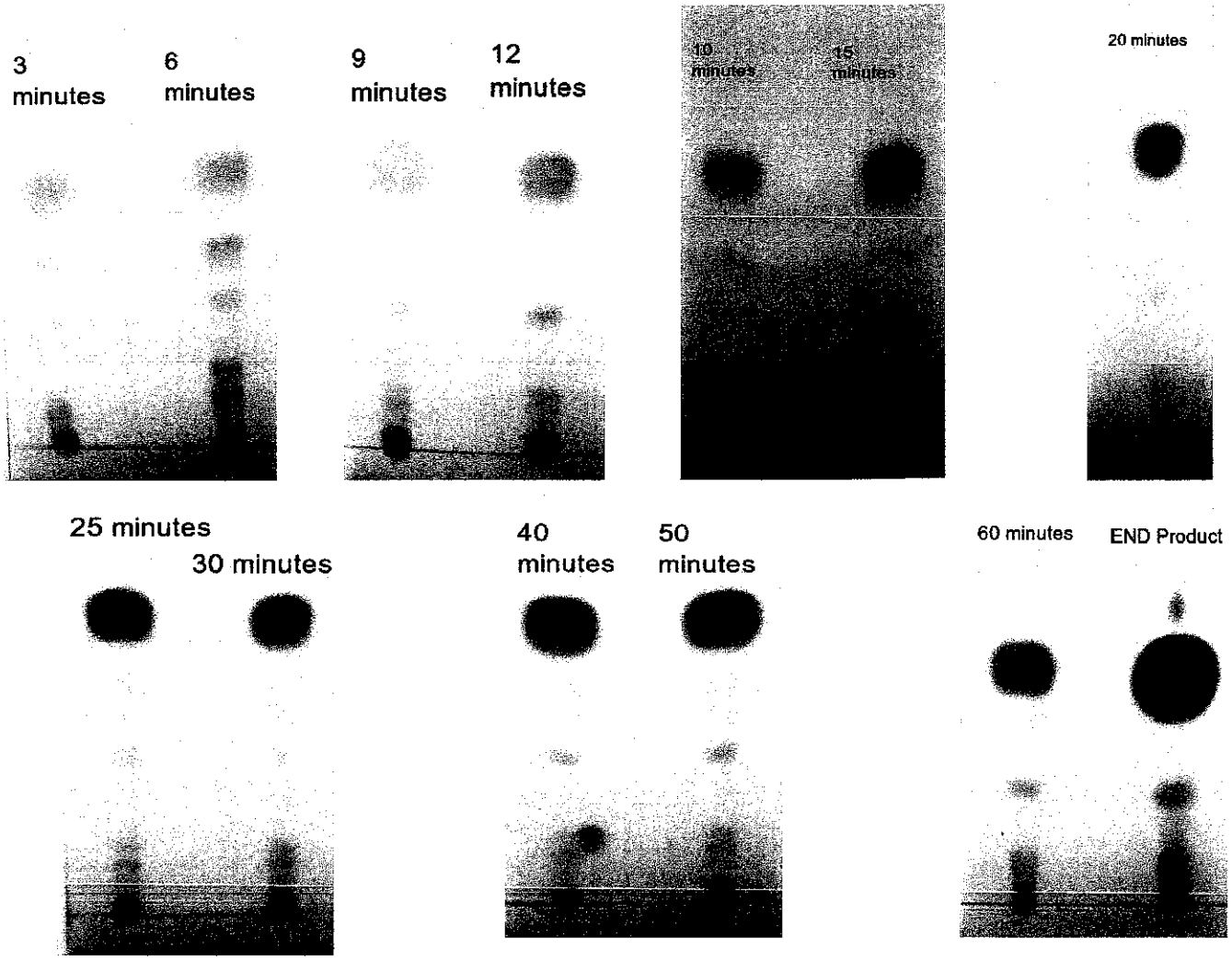
If 50 g of rapeseeds to be used, hence total oil available for extraction

$$= \frac{42}{100} \times 50 - \frac{8.5}{100} \times \left(\frac{42}{100} \times 50 \right) = 19.215g$$

For NaOH, if 0.1m catalyst was used for 100% NaOH in methanol solution (250 ml), 0.1 m built up from 0.794 g of NaOH, hence weight percentage of total catalyst used was $= \frac{0.794}{19.215} \times 100 = 4.132wt\%$

For NaOMe, if 0.1m catalyst was used for 100% NaOMe in methanol solution (250 ml), 0.1 m built up from 3.153 ml, which the mass was $m = \rho v = 0.96 \times 3.153 = 3.027g$. Therefore, weight percentage of total catalyst used was $= \frac{3.027}{19.215} \times 100 = 15.75wt\%$

8.2 TLC PLATES – TIME STUDY ANALYSIS



8.3 TLC CALIBRATION CURVE ERRORS

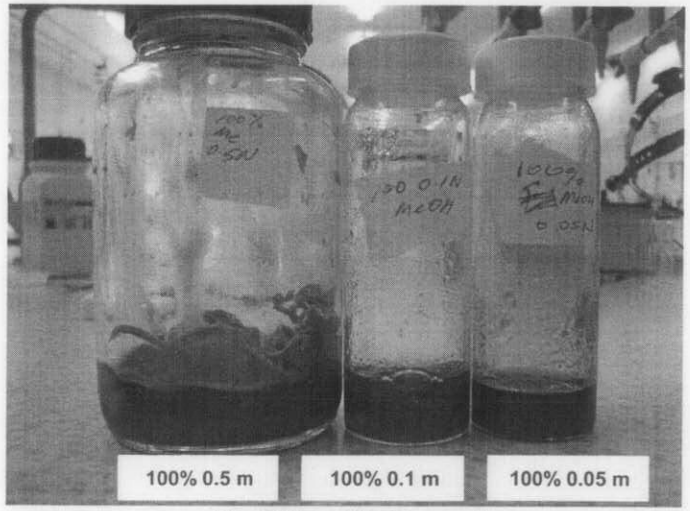
8.3.1 Ryder's Calibration Curve Errors

Biodiesel %	Area Fractions			Mean	Random Error
	Image 1	Image 2	Image 3		
0	0	0	0	0	0
11	0.181	0.140	-	0.160	0.029
31	0.453	0.473	0.433	0.453	0.020
46	0.537	0.528	0.549	0.538	0.011
66	0.596	0.620	0.567	0.594	0.026
85	0.655	0.631	0.661	0.649	0.016

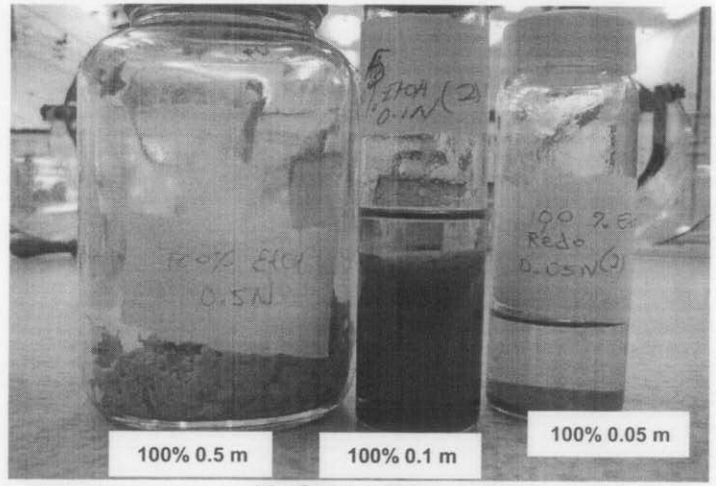
8.3.2 Modified Calibration Curve Errors

Biodiesel %	Area Fractions		Mean	Random Error
	Image 1	Image 2		
0	0	0.000	0	0
20	0.330	0.341	0.335	0.008
40	0.479	0.517	0.498	0.027
50	0.610	0.630	0.620	0.014
60	0.694	0.671	0.682	0.016
80	0.786	0.775	0.780	0.007

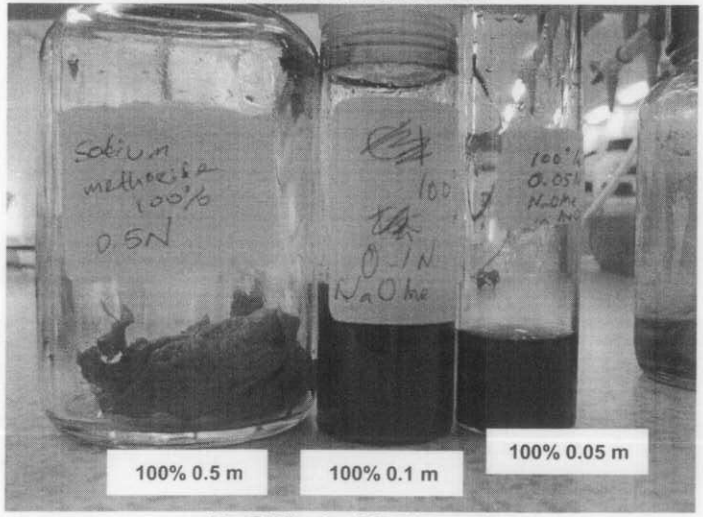
8.4 EXPERIMENTAL IMAGES



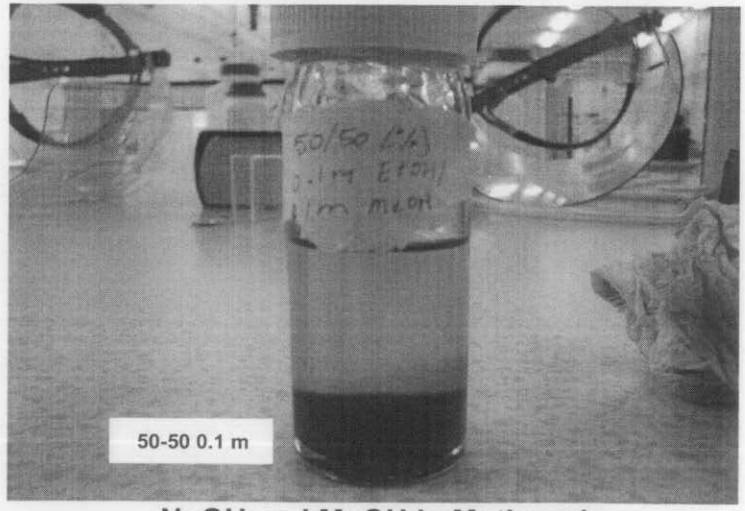
NaOH in Methanol



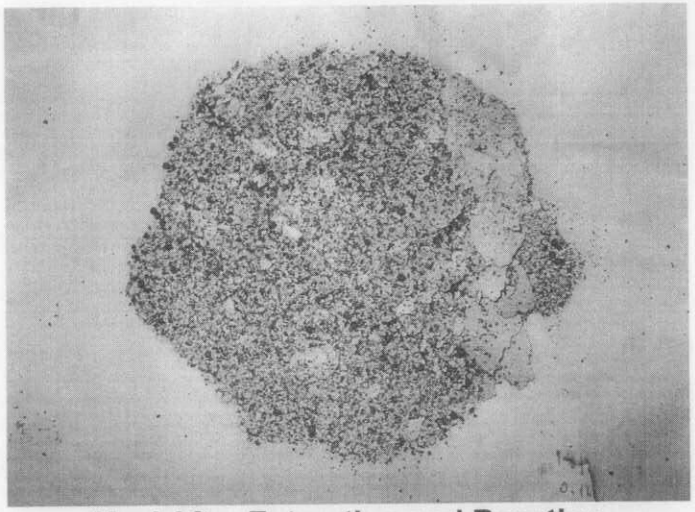
NaOH in Ethanol



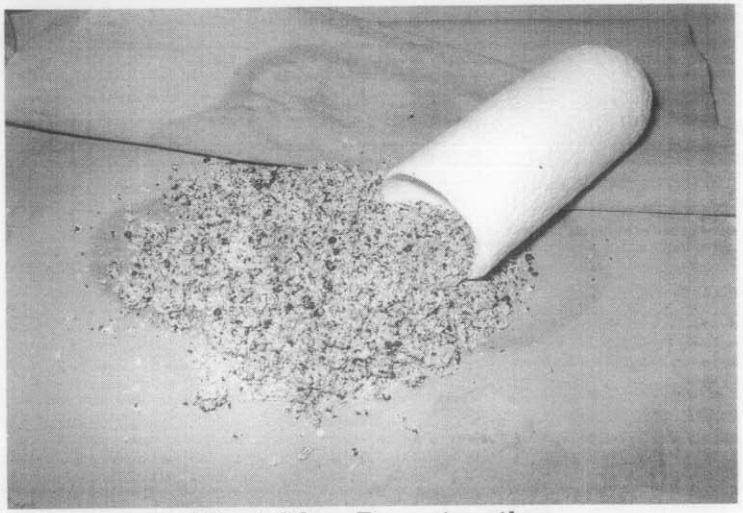
NaOMe in Methanol



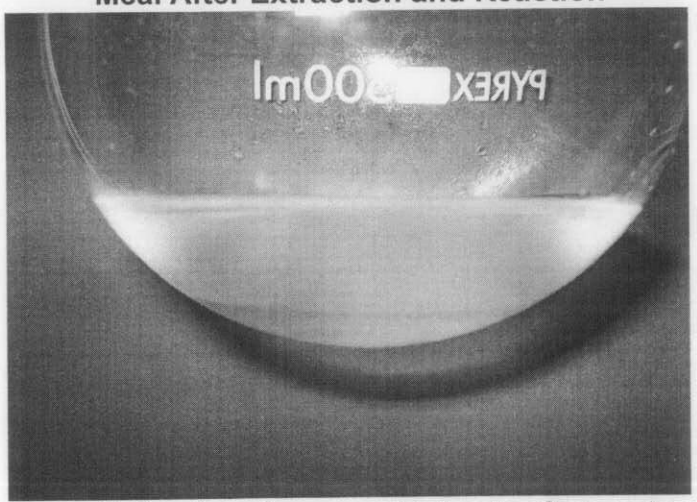
NaOH and MeOH in Methanol



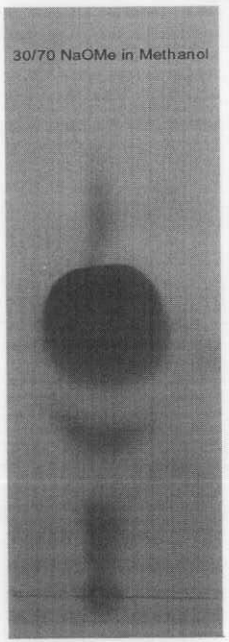
Meal After Extraction and Reaction



Meal After Re-extraction



Two distinctive layers obtained after rotary evaporation for low ratio of methanol to hexane indicated that there was no methanol adsorbed during extraction and reaction



This is the plate which is related to the left image (solvent separated from rotary evaporation unit). No biodiesel has been produced and instead of being adsorbed, methanol was fully recovered during rotary evaporation.